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OLIGONUCLEOTIDE SYNTHESIS IN TERMS OF A NOVEL TYPE OF POLYMER-SUPPORT: A CELLULOSE ACETATE FUNCTIONALIZED WITH 4-(2-HYDROXYETHYLSULFONYL)DIHYDROCINNAMOYL SUBSTITUENT[1]

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Abstract: The title polymer-support was proved to be useful practically in oligonucleotide synthesis which is characterized by the points of excellence involved in both liquid- and solid-phase approaches, i.e., being able to perform a nucleotide and/or oligonucleotide unit introduction in pyridine in a homogeneous state and to isolate the resulting polymer-support bearing the resulting oligonucleotide chain by pouring the resulting solution into an excess volume of ethanol, exemplified by an undecaribonucleotide (300 A_{260} units) and an octa-2'-deoxyribonucleotide (250 A_{260} units) syntheses.

INTRODUCTION

Since Letsinger[2] first introduced the solid-phase procedure in the field of oligonucleotide synthesis, there have been a series of attempts at improving some of the problems associated with this technology such as requiring an excess protected nucleotide unit and coupling reagents, affording a statistical mioxture of oligomers, and difficulties associated with preparative scale synthesis. The liquid-phase approach is also well known to provide oligonucleotides in a preparative scale, but it requires a chromatographic purification for each step of a series of coupling reactions and it is practically feasible only up to the synthesis of tri- and/or tetranucleotides.

Such background gave us an impetus to attempt at developing a methodology involving

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both of the merits of liquid- and solid-phase approaches, by the use of a straight chain polystyrene which is easily soluble in pyridine but insoluble in water.[3,4] If this kind of approach were feasible, it would be possible to perform an oligomer-unit-introduction (such as a hexamer unit) on to a polymer-support, which inevitably brings about much more simplified HPL chromatograms. Hayatsu and Khorana[3] and Cramer et al.[4] were, however, unsuccessful to extend such a significant methodology to the synthesis of oligo-2'-deoxyribonucleotides beyond a trimer, due to the unexpected difficulty of complete removal of an uncoupled nucleotide derivative which co-precipitated with polystyrene-polymer-support even after repetition of the dissolution-sedimentation procedure.

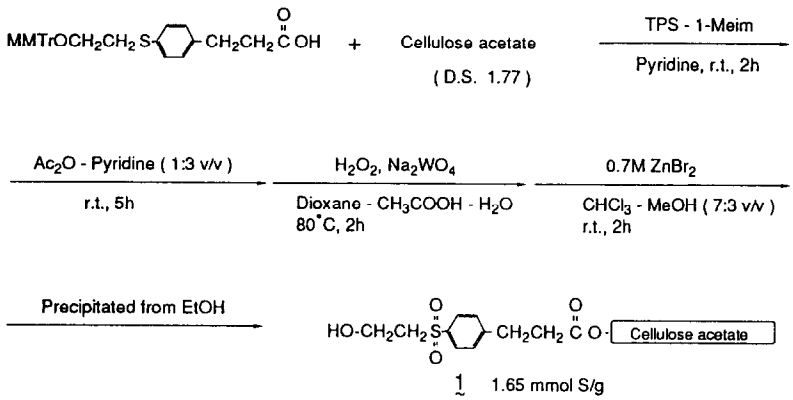
On the other hand, gene or protein engineering has advanced conspicuously, does not necessarily require a highly pure preparation of an oligonucleotide sequence, and it is feasible even if it is a statistic mixture of oligonucleotides, prepared by the solid-phase polymer-support approach, followed by the ligation processes leading to an intended gene.[5] Recent advance in this area simply require a pure sample of "primer" (ca. 20-mers), which makes enzymes to construct an objective entire gene.[6,7] In addition to this, demands are getting stronger to provide us with highly pure oligonucleotides regardless of their structures, RNA- or DNA-type, in an amount enough for the investigation on intermolecular recognition of nucleic acids with proteins by NMR spectroscpy for exapmle. The necessity to develop a more efficient and accurate synthetic approach to further highly pure oligonucleotides is therefore evident.

As recently communicated,[8] we developed a cellulose acetate derivative functionalized with 4-(2-hydroxyethylsulfonyl)dihydrocinnamoyl substituent as a novel polymer-support (**1**) for both RNA- and DNA-type oligonucleotides, which is expected to be endowed with the merits of the liquid- and solid-phase approaches.[3,4] We now report our results pertaining to the syntheses of an undecamer of oligoribonucleotide AAAAAAUUAUG (**20**) and an octamer of oligo-2'-deoxyribonucleotide dGGAATTCC (**25**).

RESULTS AND DISCUSSION

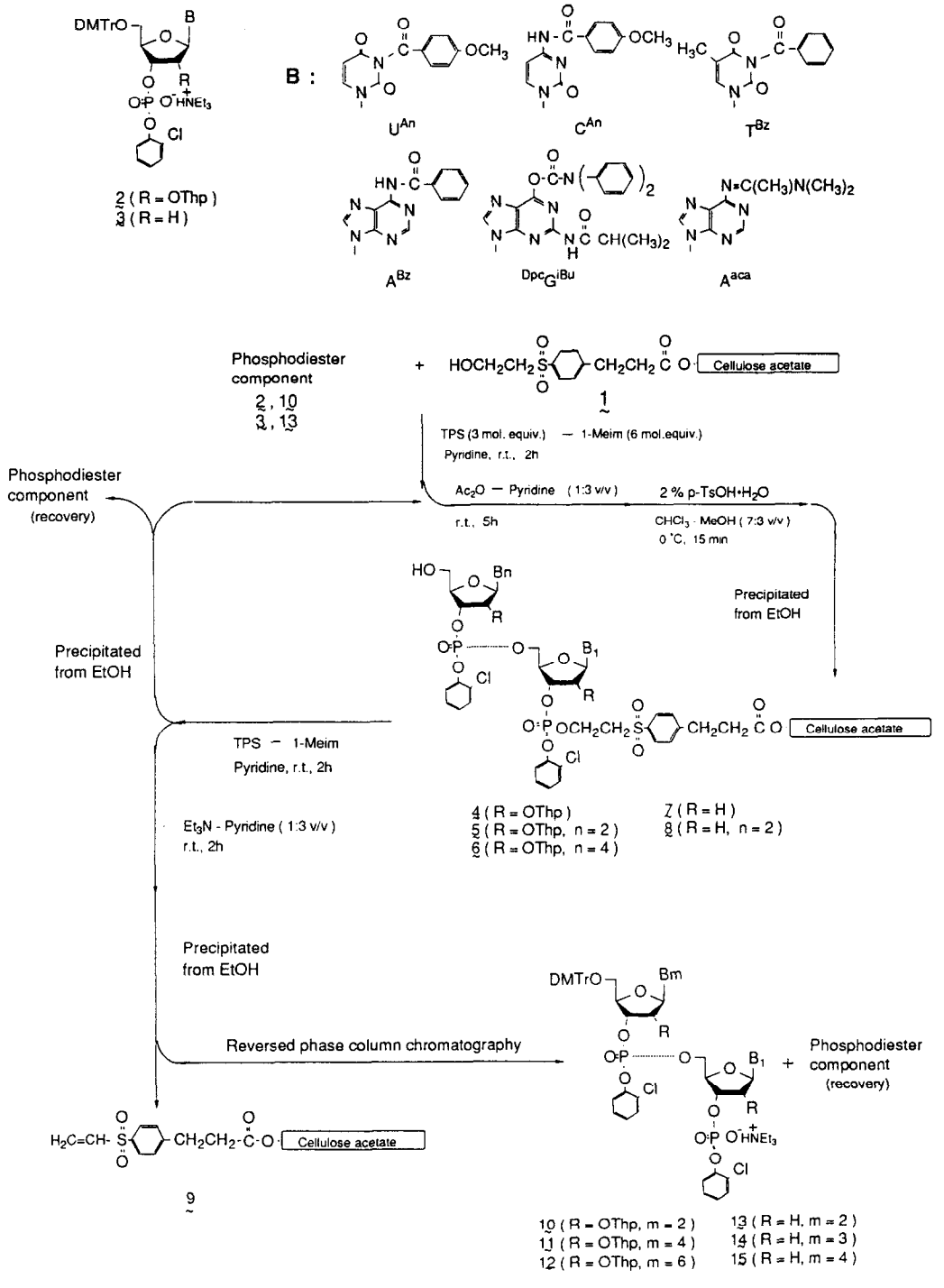
Preparation of the **1**: A cellulose acetate [Dgree of substitution (D.S.): 1.77][9] was worked up with the sequence of treatments to give **1**, i.e., coupling reaction of 2-chloro-

ethyl monomethoxytrity ether, which was prepared by monomethoxytritylation of 2-chloroethanol with monomethoxytrityl chloride (MMTrCl) in pyridine, with 4-mercaptodihydrocinnamic acid[10] in ethanol in the presence of N-ethyldiisopropylamine. Next came the coupling reaction of 4-(2-monomethoxytrityloxyethylthio)dihydrocinnamic acid with the cellulose acetate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) - 1-methylimidazole (1-Meim), capping of unchanged hydroxyl groups with acetic anhydride - pyridine, oxidation of the sulfide into the corresponding sulfone with hydrogen peroxide - sodium tungstenate (Na_2WO_4), demonomethoxytritylation with zinc bromide,[11] and, finally, precipitation from ethanol as shown in Scheme 1.[12]



Scheme 1

Synthesis of an Undecamer, AAAAAAUUAUG (**20**): A study of a leader sequence in messenger RNAs of prokaryotes[13] revealed that the leader sequence of prokaryotic and phage mRNA interestingly had a clear pattern of GG-rich and pyrimidine-poor character centered at the region from -5 to -10 (i.e., the Shine-Dalgarno sequence), which might be deeply associated with the efficiency of biosynthesis of proteins. The assumption led us to synthesize the undecamer **20** corresponding to the AAGGAAAAUUAUG sequence built in T4rIIB mRNA.[14] The strategy for the synthesis of **20** is as shown in Scheme 2. The synthesis of **20** was initiated from triethylammonium diribonucleotides 3'-phosphodiesters (**10**) by the use of **1** and triethylammonium 5'-O-dimethoxytrityl-2'-O-(tetrahydropyran-2-yl)ribonucleosides 3'-(2-chlorophenyl)phosphates (**2**). Thus, introduction of the monomer units (**2**)


DMTrO
B
R
O-P-O⁻HNEt₃
Cl
2 (R = OThp)
3 (R = H)
B :
U^An
C^An
T^Bz
A^Bz
DpcG^iBu
A^aca
Phosphodiester component
2, 10
3, 13
HOCH₂CH₂S
CH₂CH₂CO
Cellulose acetate
1
TPS (3 mol. equiv.) — 1-Meim (6 mol.equiv.)
Pyridine, r.t., 2h
Phosphodiester component (recovery)
Ac₂O — Pyridine (1:3 v/v)
r.t., 5h
2 % p-TsOH·H₂O
CHCl₃ - MeOH (7:3 v/v)
0 °C, 15 min
Precipitated from EtOH
HO
Bn
B₁
TPS — 1-Meim
Pyridine, r.t., 2h
Et₃N - Pyridine (1:3 v/v)
r.t., 2h
4 (R = OThp)
5 (R = OThp, n = 2)
6 (R = OThp, n = 4)
7 (R = H)
8 (R = H, n = 2)
Precipitated from EtOH
Reversed phase column chromatography
DMTrO
Bm
Phosphodiester component (recovery)
H₂C=CH-SO₂-CH₂CH₂-CO-O
9
10 (R = OThp, m = 2)
11 (R = OThp, m = 4)
12 (R = OThp, m = 6)
13 (R = H, m = 2)
14 (R = H, m = 3)
15 (R = H, m = 4)

Scheme 2

Table 1. Synthesis of Diribonucleotide 3'-Phosphodiester Derivatives (10) by the Use of 1 as a Novel Polymer-support

Step 1 ; Introduction of Mononucleotide Unit onto 1

Entry	Polymer-support 1 mmol/g of the loaded spacer	(g, mmol)	2 B_1	(g, mmol)	TPS (g, mmol)	1-Meim (mL, mmol)	Yield of 4 B_1	g	(mmol,	%,	mmol/g[a])
1	1.65	(0.2424, 0.4)	U^{An}	(0.3170, 0.9)	0.2726, 0.9	0.14, 1.8	U^{An}	0.3330	(0.224,	75,	0.674)
2	1.65	(0.2424, 0.4)	U^{An}	(0.3170, 0.9)	0.2726, 0.9	0.14, 1.8	U^{An}	0.3680	(0.255,	85,	0.693)
3	1.65	(0.6061, 1.0)	A^{Bz}	(0.7872, 0.75)	0.6814, 2.25	0.36, 4.5	A^{Bz}	0.9166	(0.638,	85,	0.696)

[a] Loaded amount of mononucleotides

Step 2; Syntheses of 10

Entry	2 B_2	(g, mmol)	4 B_1	(g, mmol)	TPS (g, mmol)	1-Meim (mL, mmol)	Yield of 10 (m = 2) B_1	B_2	g	(mmol, %)
1	U^{An}	(0.2958, 0.28)	U^{An}	(0.3330, 0.224)	0.2544, 0.84	0.13, 1.68	U^{An}	U^{An}	0.2926	(0.174, 58)
2	A^{Bz}	(0.3359, 0.32)	U^{An}	(0.3680, 0.255)	0.2907, 0.96	0.15, 1.92	U^{An}	A^{Bz}	0.2978	(0.177, 59)
3	A^{Bz}	(0.8370, 0.80)	A^{Bz}	(0.9166, 0.638)	0.7269, 2.40	0.38, 4.80	A^{Bz}	A^{Bz}	0.7701	(0.458, 61)

Table 2. Synthesis of Tetraribonucleotide 3'-Phosphodiester Derivative (11) by the Use of 1

Step 1; Introduction of Dinucleotide Unit, 10 (n = 2; $B_1 = B_2 = A^{Bz}$), onto 1

10 (g, mmol)	1 mmol/g of the loaded spacer	(g, mmol)	TPS (g, mmol)	1-Meim (mL, mmol)	Yield of 5 (n = 2; $B_1 = B_2 = A^{Bz}$) g	(mmol,	%,	mmol/g[a])
0.5871, 0.35	1.65	(0.3182, 0.525)	0.3180, 1.05	0.17, 2.10	0.6248	(0.289,	82,	0.462)

Step 2; Synthesis of 11

10 (n = 2; $B_1 = B_2 = A^{Bz}$) (g, mmol)	5 (g, mmol)	TPS (g, mmol)	1-Meim (mL, mmol)	Yield of 11 (m = 4; $B_1 = B_2 = B_3 = B_4 = A^{Bz}$) g	(mmol,	%)
0.6760, 0.403	0.6248, 0.289	0.3662, 1.21	0.20, 2.42	0.5095	(0.175,	50)

[a] Loaded amount of dinucleotide

Table 3. Synthesis of Hexaribonucleotide 3'-Phosphodiester Derivative (12) by the Use of 1

Step 1; Introduction of Dinucleotide Unit, 10 (n = 2 ; $B_1 = U^{An}$, $B_2 = A^{Bz}$), onto 1

10	1 mmol/g of the loaded		TPS	1-Meim	Yield of 5 (n = 2; $B_1 = U^{An}$, $B_2 = A^{Bz}$)	
(g, mmol)	spacer	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %, mmol/g*)
0.5048, 0.30	1.65	(0.2727, 0.45)	0.2726, 0.90	0.14, 1.80	0.5090	(0.217, 72, 0.427)

Step 2; Introduction of Dinucleotide Unit, 10 (n = 2; $B_1 = B_2 = U^{An}$), onto 1 after the Step 1

10	5 (n = 2; $B_1 = U^{An}$, $B_2 = A^{Bz}$)	TPS	1-Meim	Yield of 6 (n = 4; $B_1 = U^{An}$, $B_2 = A^{Bz}$, $B_3 = B_4 = U^{An}$)	
(g, mmol)	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %, mmol/g*)
0.5526, 0.326	0.5090, 0.217	0.2962, 0.98	0.16, 1.96	0.5173	(0.141, 47, 0.273)

Step 3; Synthesis of 12

10	6 (n = 4; $B_1 = U^{An}$, $B_2 = A^{Bz}$, $B_3 = B_4 = U^{An}$)	TPS	1-Meim	Yield of 12 (m = 6; $B_1 = U^{An}$, $B_2 = A^{Bz}$, $B_3 = B_4 = U^{An}$, $B_5 = B_6 = A^{Bz}$)	
(g, mmol)	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %)
0.3523, 0.21	0.5173, 0.141	0.1908, 0.63	0.10, 1.26	0.2527	(0.063, 21)

* Loaded amount of dinucleotides

onto **1** mediated by TPS and 1-Meim in pyridine, followed by capping with acetic anhydride, dedimethoxytritylation, and sedimentation from ethanol, gave the derivatives of **1** loading the monomer units (**4**), respectively. The conditions used and the results obtained are summarized in Step 1 of Table 1. The resulting **4**s were subjected to the second step of introducing monomer units **2** in the same way as above to give the derivatives of **1** loading dimer units (**5**; n = 2). These were then subjected to a splitting-off procedure by treatment with 1:3 triethylamine - pyridine (β-elimination), to give the corresponding dimer units (**10**; m = 2). The conditions used and the results obtained are summarized in Step 2 of Table 1. Tetraribonucleotide unit (**11**; m = 4; $B_1 = B_2 = B_3 = B_3 = A^{Bz}$) and hexamer unit (**12**; m = 6; $B_1 = U^{An}$, $B_2 = A^{Bz}$, $B_3 = B_4 = U^{An}$, $B_5 = B_6 = A^{Bz}$) were similarly synthesized in the manner of dimer units introduction; the conditions used and the results obtained are summarized in Table 2 and 3, respectively.

The synthesis of **18** was achieved by the successive introductions of **12** and **11** onto the derivative of **1**, loading O^6-diphenylcarbamoyl-N^2-isobutyryl-2'-O-(tetrahydropyran-2-

yl)guanosine 3'-[3-(carboxy)propionate] function (**16**) in the same way as above described. The splitting-off of the undecamer unit from **18** [ca. 1/200 of the product (69% yield) was used] through 0.5 M N^1,N^1,N^3,N^3-tetramethylguanidium (Z)-2-pyridinealdoximate (TMG·PAO) affording $DMTrA^tpA^tpA^tpA^tpA^tpA^tpU^tpU^tpA^tpU^tpG^t$ (**19**) (See Fig. 1), followed by the treatment of **19** with hydrochloric acid (pH 2), gave **20** (1.51 A_{260} units) (See Figs. 2-I, 2-II, and Scheme 3). Compound **20** afforded a clear electrophoretic band (See Fig. 3) on 20% polyacrylamide gel containing 7 M urea after 5'-O-[^{32}P]-phosphorylation according to the method reported by Miura et al.[15] After complete degradation of **20** by nuclease P1 the HPL chromatogram (See Fig. 4) proved the composition of ribonucleotides units as expected for **20**.

Synthesis of an Octamer, dGGAATTCC (**25**): The successful undecamer synthesis by the above described appraoch prompted us to extend our studies to the synthesis of the title octamer known as one of the typical "linker."[16]

The synthesis of **25** was achieved as described for **20** (Schema 3 and 4). A sequence of reactions, involving a monomer unit introduction onto **1**, capping, and dedimethoxytri-

Table 4. Synthesis of Di-2'-deoxyribonucleotide 3'-Phosphodiester Derivatives (13) by the Use of 1

Step 1; Introduction of Mono 2'-deoxyribonucleotide Unit onto 1

Entry	1 mmol/g of the loaded spacer	3 (g, mmol) B_1	TPS (g, mmol)	1-Meim (g, mmol)	Yield of 7 B_1	g	(mmol, %, mmol/g[a])
1	1.30	(0.4965, 0.65) T^{Bz} (0.4710, 0.65)	0.4550,0.15	0.23,3.0	T^{Bz}	0.4725	(0.292, 70, 0.618)
2	1.65	(0.2424, 0.40) $^{Dpc}G^{iBu}$ (0.3370,0.30)	0.2726,0.90	0.14,1.8	$^{Dpc}G^{iBu}$	0.4105	(0.260, 87, 0.635)
3	1.65	(0.6280, 1.04) A^{aca} (0.7910,0.86)	0.7850,2.59	0.41,5.2	A^{aca}	1.0490	(0.840, 97, 0.801)

[a] Loaded amount of mono-2'-deoxyribonucleotides

Step 2; Syntheses of 13

Entry	3 B_1 (g, mmol)	7 B_2 (g, mmol)	TPS (g, mmol)	1-Meim (mL, mmol)	Yield of 13 (m = 2) B_1	B_2	g	(mmol, %)
1	T^{Bz} (0.3300,0.35)	T^{Bz} (0.4725,0.29)	0.3115,1.03	0.16, 2.0	T^{Bz}	T^{Bz}	0.4610	(0.315, 63)
2	$^{Dpc}G^{iBu}$ (0.3700,0.33)	$^{Dpc}G^{iBu}$ (0.4105,0.26)	0.2990,0.99	0.16 , 2.0	$^{Dpc}G^{iBu}$	$^{Dpc}G^{iBu}$	0.2790	(0.153, 51)
3	A^{aca} (0.6400,0.70)	A^{aca} (1.0490,0.80)	0.5820,1.92	0.33, 3.9	A^{aca}	A^{aca}	0.5800	(0.406, 47)

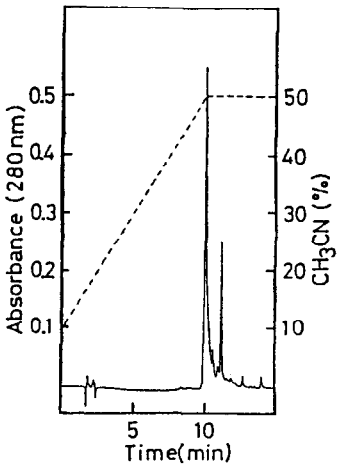


Fig. 1.

Reversed-phase HPLC of the undecamer bearing 2'-O-THP and 5'-terminal O-DMTr Groups [Condition: column of M&S PACK C18 (4.6 mm ID x 150 mm L); mobile phase 5% acetonitrile in 0.1 M TEAA pH 7 (buffer A) and 50% acetonitrile in 0.1 M TEAA pH 7 (buffer B); buffer composition 10 – 100% B, changing the composition by 9% min^{-1}; 100% B after the passage of 10 min; flow rate 1 mL min^{-1}; detected by u.v. at 280 nm].

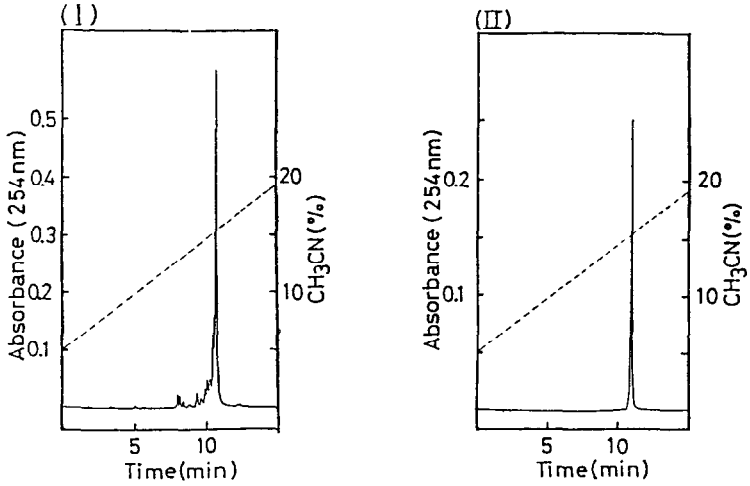
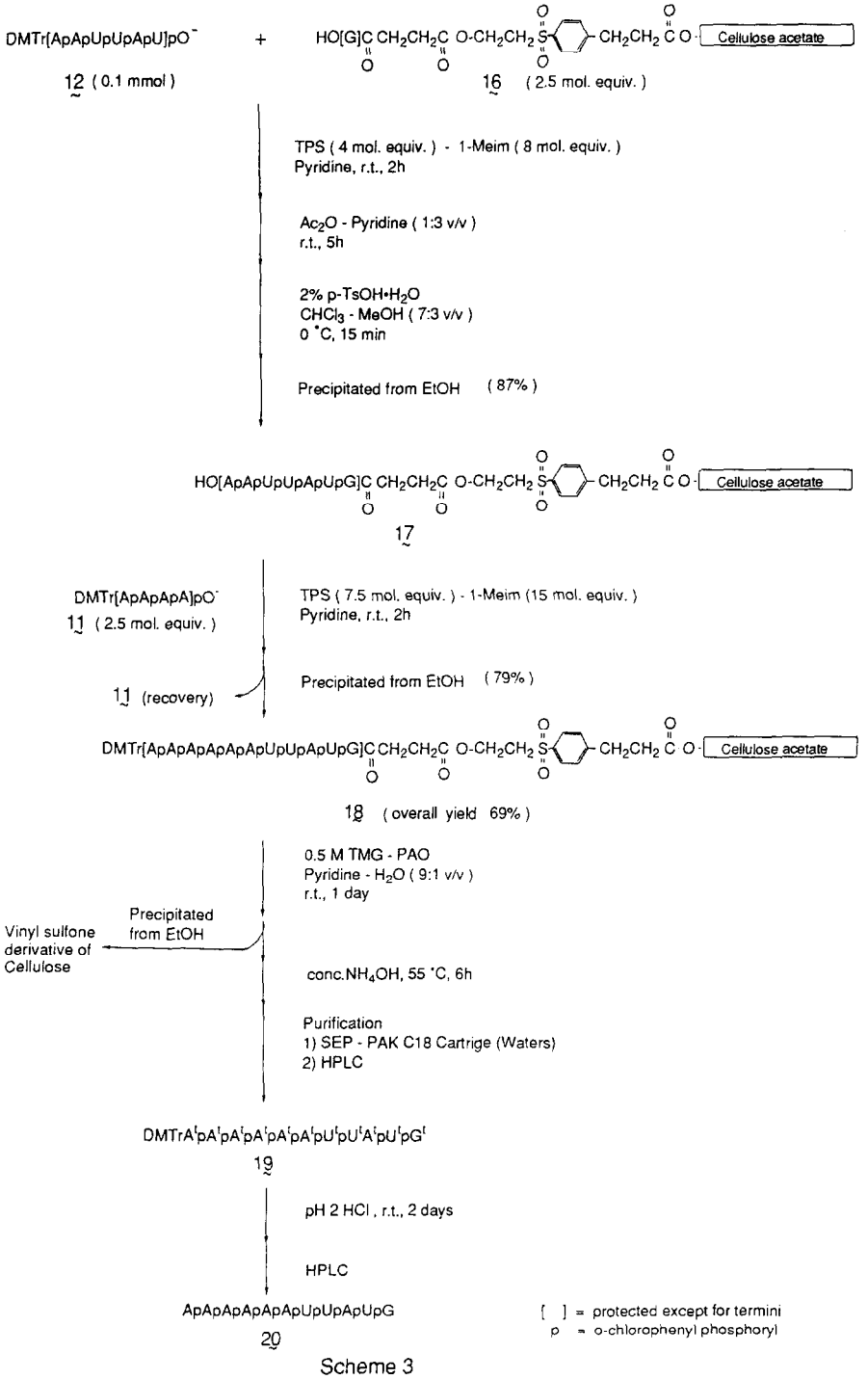


Fig. 2.

Conditions: column of M&S PACK C18 (4.6 mm ID x 150 mm L); mobile phase 5% CH_3CN in 0.1 M TEAA pH 7 (buffer A) and 25% CH_3CN in 0.1 M TEAA pH 7 (buffer B); buffer composition 0 – 70%, composition changing by 4.7% min^{-1}; flow rate 1 mL min^{-1}; detected by u.v. at 254 nm.

(I) Reversed-phase HPLC of the mixture obtained by removal of DMTr and THP protecting groups at pH 2 (aq. HCl).

(II) Reversed-phase HPLC of the free undecamer isolated from the mixture of (I).


DMTr[ApApUpUpApU]pO⁻
12 (0.1 mmol)
+
HO[G]C CH2CH2C O-CH2CH2 S CH2CH2 C O · Cellulose acetate
16 (2.5 mol. equiv.)
TPS (4 mol. equiv.) - 1-Meim (8 mol. equiv.)
Pyridine, r.t., 2h
Ac2O - Pyridine (1:3 v/v)
r.t., 5h
2% p-TsOH•H2O
CHCl3 - MeOH (7:3 v/v)
0 °C, 15 min
Precipitated from EtOH (87%)
HO[ApApUpUpApUpG]C CH2CH2C O-CH2CH2 S CH2CH2 C O · Cellulose acetate
17
DMTr[ApApApA]pO⁻
11 (2.5 mol. equiv.)
TPS (7.5 mol. equiv.) - 1-Meim (15 mol. equiv.)
Pyridine, r.t., 2h
11 (recovery)
Precipitated from EtOH (79%)
DMTr[ApApApApApApUpUpApUpG]C CH2CH2C O-CH2CH2 S CH2CH2 C O · Cellulose acetate
18 (overall yield 69%)
0.5 M TMG - PAO
Pyridine - H2O (9:1 v/v)
r.t., 1 day
Precipitated from EtOH
Vinyl sulfone derivative of Cellulose
conc.NH4OH, 55 °C, 6h
Purification
1) SEP - PAK C18 Cartrige (Waters)
2) HPLC
DMTrA^tpA^tpA^tpA^tpA^tpA^tpU^tpU^tA^tpU^tpG^t
19
pH 2 HCl , r.t., 2 days
HPLC
ApApApApApApUpUpApUpG
20
[ ] = protected except for termini
p = o-chlorophenyl phosphoryl

Scheme 3

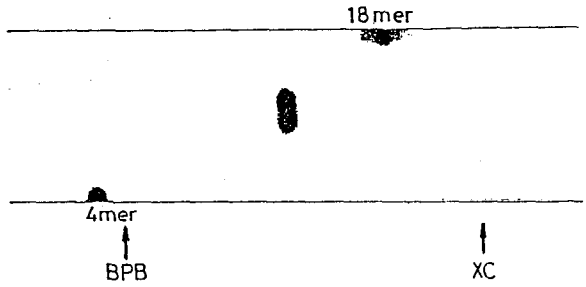
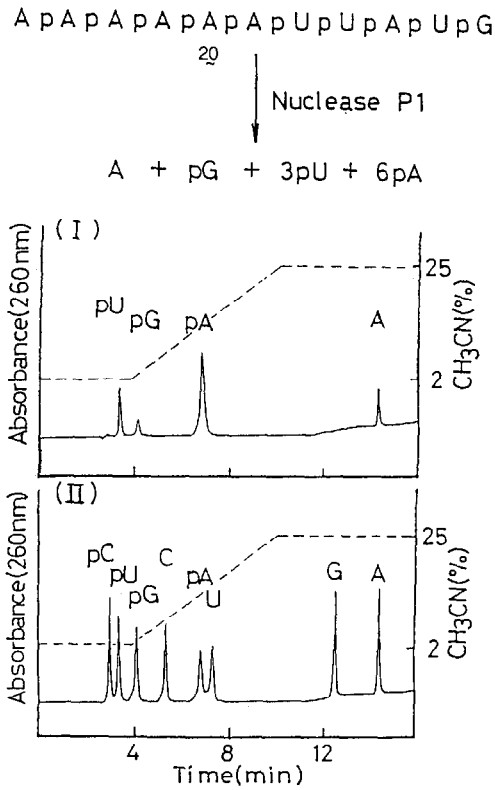


Fig. 3.
Electrophoresis of the Undecamer (^{32}pApApApApApApUpUpApUpG) on 20% Polyacrylamide Gel Containing 7 M Urea after 5'-O-Phosphorylation Using (γ-^{32}p)ATP and T4 Polynucleotide Kinase

Reversed-phase HPLC Analysis of Products Obtained by Digestion of the Completely Unmasked Undecamer with Nuclease P1 (I), and of Standard Samples (II)



Conditions: column of M&S PACK C18 (4.6 mm ID x 150 mm L); mobile phase 2% CH_3CN in 0.05 M $NH_4H_2PO_4$ pH 5.2 (buffer A) and 25% CH_3CN in 0.05 M $NH_4H_2PO_4$ pH 5.2; buffer composition 0 – 50% B, 2% B up to 4 min, changing the composition by 8.3% min^{-1} from 4 to 10 min, 25% B after the passage of 10 min; flow rate 1 mL min^{-1}; detected by u.v. at 260 nm.

Fig. 4

tylation affording **7**, introduction of the second monomer unit affording **8**, and splitting-off of a dimer unit loaded on **13** (m = 2), gave the results as summarized in Steps 1 and 2 of Table 4. The trimer (**14**; m = 3; $B_1 = C^{An}$, $B_2 = B_3 = T^{Bz}$) was synthesized by the introduction of the dimer unit (**13**; m = 2; $B_1 = B_2 = T^{Bz}$) onto **7** ($B_1 = C^{An}$) as shown Scheme 2; the conditions used and the results obtained are summarized in Steps 1 and 2 of Table 5.

Table 5. Synthesis of Tri-2'-deoxyribonucleotide 3'-Phosphodiester Derivative (14) by the Use of 1

Step 1; Introduction of Mono-2'-deoxyribonucleotide Unit, 3 ($B_1 = C^{An}$), onto 1

3	1		TPS	1-Meim	Yield of 7 ($B_1 = C^{An}$)	
	mmol/g of the loaded					
(g, mmol)	spacer	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %, mmol/g[a])
0.9550, 1.0	1.61	(0.8150, 1.39)	0.8800, 2.90	0.48, 6.0	0.9370	(0.64, 64, 0.682)

Step 2; Snthesis of 14

13	7	TPS	1-Meim	Yield of 14	
($n = 2; B_1 = B_2 = T^{Bz}$)				($m = 3; B_1 = C^{An}, B_2 = B_3 = T^{Bz}$)	
(g, mmol)	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %)
0.7560, 0.52	0.9370, 0.64	0.4840, 1.60	0.26, 3.2	0.5180	(0.25, 47)

[a] Loaded amount of mono-2'-deoxyribonucleotide

Table 6. Synthesis of Tetra-2'-deoxyribonucleotide 3'-Phosphodiester Derivative (15) by the Use of 1

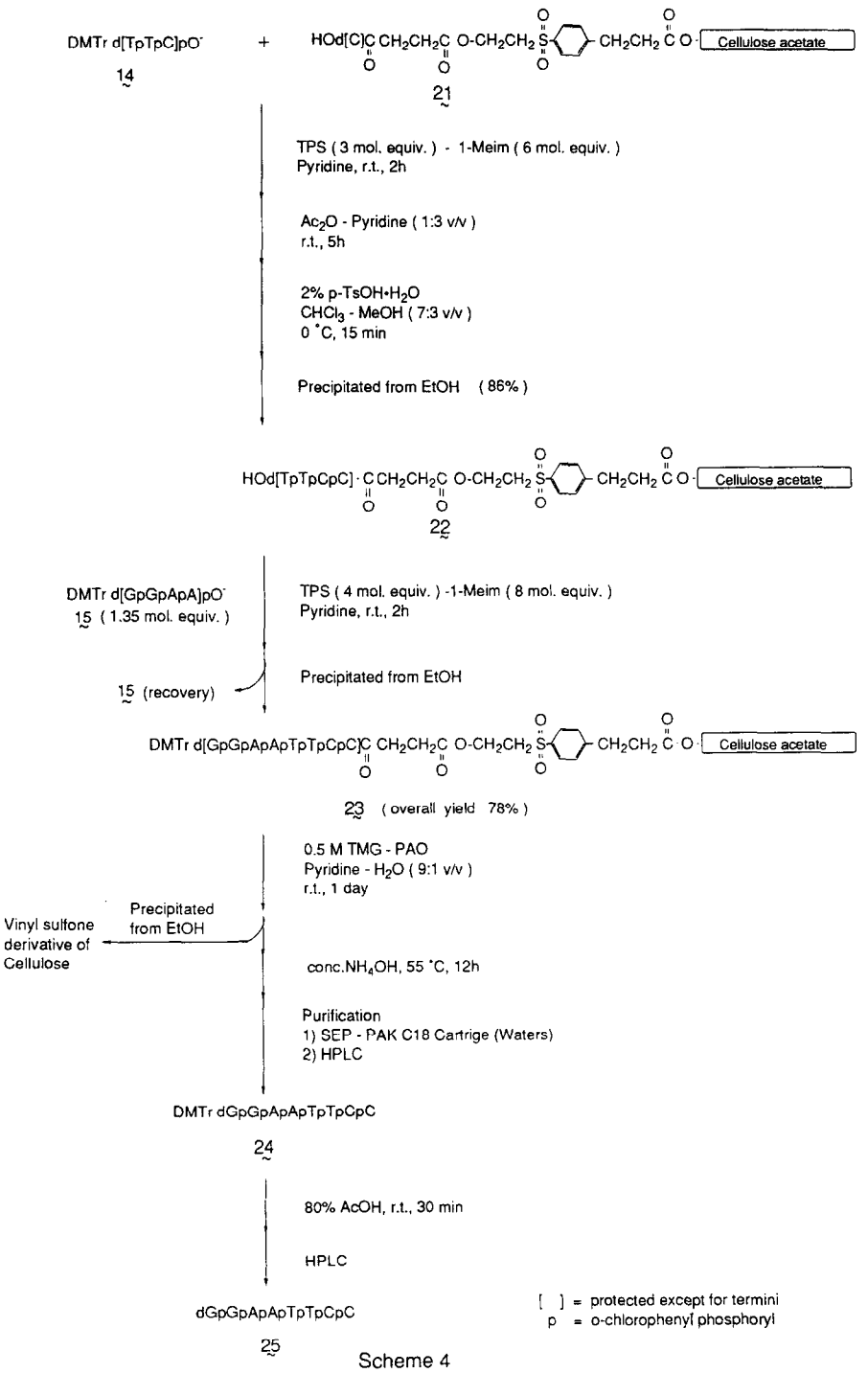
Step 1; Introduction of Di-2'-deoxyribonucleotide Unit, 13 (n = 2; $B_1 = B_2 = A^{Bz}$), onto 1

13	1		TPS	1-Meim	Yield of 8 (n = 2; $B_1 = B_2 = A^{Bz}$)	
	mmol/g of loaded					
(g, mmol)	spacer	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %, mmol/g[a])
0.614o, 0.40	1.61	(0.3750, 0.61)	0.3660, 1.21	0.20, 2.40	0.7720	(0.306, 76, 0.396)

Step 2; Synthesis of 15

13	8	TPS	1-Meim	Yield of 15	
($n = 2; B_1 = B_2 = {}^{Dpc}G^{Bu}$)				($m = 4; B_1 = B_2 = A^{Bz}, B_3 = B_4 = {}^{Dpc}G^{Bu}$)	
(g, mmol)	(g, mmol)	(g, mmol)	(mL, mmol)	g	(mmol, %)
0.6710, 0.36	0.7720, 0.31	0.3330, 1.10	0.18, 2.2	0.6000	(0.21, 69)

[a] Loaded amount of di-2'-deoxyribonucleotide


DMTr d[TpTpC]pO⁻
14
HOd[C]C CH2CH2C O-CH2CH2 S CH2CH2 C O · Cellulose acetate
21
TPS (3 mol. equiv.) - 1-Meim (6 mol. equiv.)
Pyridine, r.t., 2h
Ac2O - Pyridine (1:3 v/v)
r.t., 5h
2% p-TsOH•H2O
CHCl3 - MeOH (7:3 v/v)
0 °C, 15 min
Precipitated from EtOH (86%)
HOd[TpTpCpC] · C CH2CH2C O-CH2CH2 S CH2CH2 C O · Cellulose acetate
22
DMTr d[GpGpApA]pO⁻
15 (1.35 mol. equiv.)
TPS (4 mol. equiv.) -1-Meim (8 mol. equiv.)
Pyridine, r.t., 2h
15 (recovery)
Precipitated from EtOH
DMTr d[GpGpApApTpTpCpC] C CH2CH2C O-CH2CH2 S CH2CH2 C O · Cellulose acetate
23 (overall yield 78%)
0.5 M TMG - PAO
Pyridine - H2O (9:1 v/v)
r.t., 1 day
Precipitated from EtOH
Vinyl sulfone derivative of Cellulose
conc.NH4OH, 55 °C, 12h
Purification
1) SEP - PAK C18 Cartrige (Waters)
2) HPLC
DMTr dGpGpApApTpTpCpC
24
80% AcOH, r.t., 30 min
HPLC
dGpGpApApTpTpCpC
25
[ ] = protected except for termini
p = o-chlorophenyl phosphoryl

Scheme 4

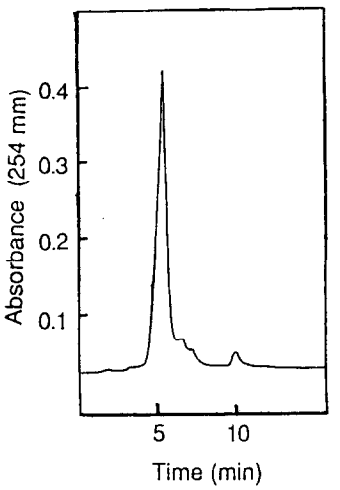


Fig. 5

Reversed-phase HPLC of the octamer bearing 5'-terminal O-DMTr Group
[Conditions; column of LiChrosorb RP-18 (4.0 mm ID x 250 mm L); mobile phase 25% CH_3CN in 0.1 M TEAA pH 7; flow rate 1 mL min^{-1}; detected by u.v. at 254 nm].

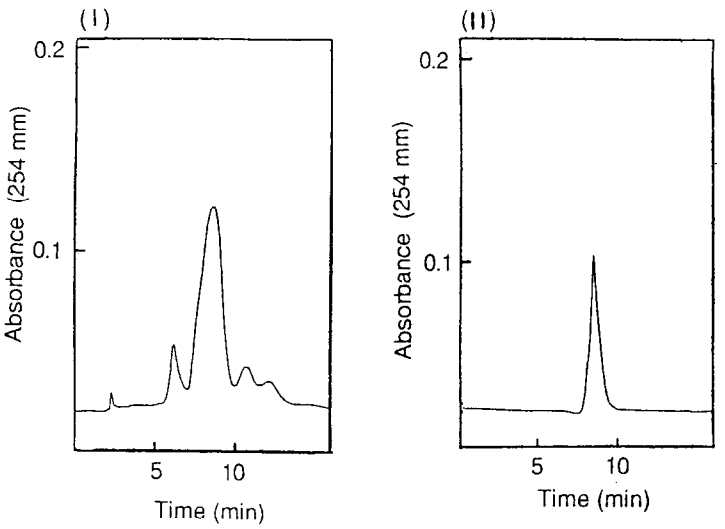


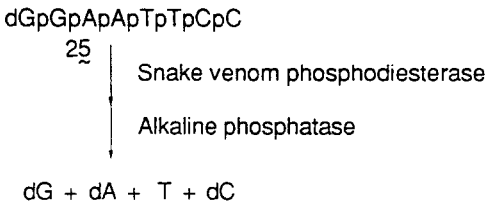
Fig. 6

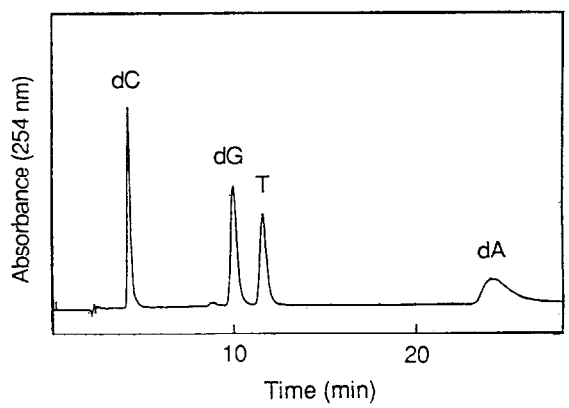
Conditions; column of LiChrosorb RP-18 (4.0mm ID x 250 mm L); mobile phase 10% CH_3CN in 0.1 M TEAA pH 7; flow rate 1 mL min^{-1} ;detected by u.v. at 254 nm.

(I) Reversed-phase HPLC of mixture obtained by removal of DMTr protecting group at 80% acetic acid.

(II) Reversed-phase HPLC of the free octamer from the mixture of (I).

Reversed-phase HPLC Analysis of Products Obtained
by Digestion of Completely Unmasked Octamer with
Snake venom phosphodiesterase and Alkaline phosphatase





Cnditions; column of LiChrosorb RP-18 (4.0 mm ID x 250 mm L); mobile phase 3% CH_3CN in 0.1 M NaH_2PO_4; flow rate 1 mL min^{-1}; detected by u.v. at 254 nm.

Fig. 7

The tetramer unit (**15**; m = 4; $B_1 = B_2 = A^{aca}$, $B_3 = B_4 = {}^{DPC}G^{iBu}$) was synthesized similarly by the introduction of a dimer unit (**13**; n = 2; $B_1 = B_2 = {}^{DPC}G^{iBu}$) onto **8** (n = 2; $B_1 = B_2 = A^{aca}$) as shown in Scheme 2. The conditions used and the results obtained are summarized in Steps 1 and 2 of Table 6.

The synthesis of **25** (See Scheme 4) was performed by the successive reactions of the trimer unit **14** and, then, the tetramer unit **15** onto the derivative of **1**, loading $\underline{N}^4$-anisoyl-2'-deoxycytidine 3'-[3-(carboxy)propionate] function (**21**), followed by a sequence of treatments with TMG•PAO in 9:1 pyridine - water, aqueous conc. ammoniacal solution, and the subsequent purification, gave DMTrdGpGpApApTpTpCpC (**24**), whose HPLC profile is shown in Fig. 5. The main peak of the mixture, after separation, dedimethoxytritylation with 80% aqueous acetic acid, and HPL chromatographic purification, gave a sample affording the HPL chromatogram of which is shown in Fig. 6-I. The main peak was separated and further purified to give a pure sample of **25**, with the HPL chromatogram shown in Fig. 6-II. Complete degradation of **25** to each component of 2'-deoxyribonucleosides was also conducted by the successive treatments with snake venom phosphodiesterase and alkaline phosphatase. The HPL chromatogram of such an experiment is shown in Fig. 7.

EXPERIMENTAL

Melting points were determined by a Yanagimoto Micro-Melting-Point apparatus, and are uncorrected. 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), or 6:4 benzene - acetone (Solvent C), and reversed phase t.l.c. was on Merck silanized gel RP18 $F_{254}S$ with 6:4 acetone - water (Solvent D) or 7:3 acetone - water (Solvent E). 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 - 230 mesh, purchased from Merck) by the use of acetone - 0.05 M triethylammonium hydrogencarbonate (bicarbonate)(TEAB). High performance liquid chromatography (HPLC) was performed on M & S PACK C-18 (4.6 mm ID x 150 mm L) for purification of the RNA-type undecamer and on Li-Chrosorb RP-18 (4 mm ID x 250 mm L) for that of the DNA-type octamer by the use of acetonitrile - 0.1 M triethylammonium acetate (TEAA; pH 7) as the eluant. ^{1}H-N.m.r. spectra were recorded on a JEOL JNM FX-200 apparatus with tetramethylsilane (TMS) as the internal standard. Elemental analyses were achieved with a Perkin-Elmer 240-002 apparatus.

2-Chloroethyl monomethoxytrityl ether: Monomethoxytrityl chloride (9.2643 g, 30 mmol) was treated with 2-chloroethanol (4.02 mL, 60 mmol) in pyridine (150 mL) at room temperature for 2 h, with stirring. The resulting mixture was quenched with chilled-water (15 mL) with stirring for 30 min. The mixture was extracted with chloroform (200 x 2 and 100 mL), and the organic layer was dried over anhydrous magnesium sulfate after washing with water (200 x 2 mL). The desiccant was filtered off and the filtrate was evaporated to give a residue, which was crystallized from hexane (100 mL) to give the ether (9.6307 g, 91% yield), m.p. 87 - 88°C (from hexane), ^{1}H-n.m.r.(chloroform-d - TMS): δ 3.30 - 3.50 (4H, m, C-C$\underline{H}_2$ x 2), 3.60 (3H, s, OC$\underline{H}_3$), 6.70 (2H, d, J 9 Hz, Ph proton x 2), 6.97 - 7.53 (12H, m, Ph

proton x 12).

Anal. Calcd for $C_{22}H_{21}O_2Cl$: C, 74.89; H, 6.00. Found: C, 75.00; H, 5.98.

4-(2-Monomethoxytrityloxyethylthio)dihydrocinnamic acid: A solution of 2-chloroethyl monomethoxytrityl ether (2.1172 g, 6 mmol) and 4-mercaptodihydrocinnamic acid[10](0.9112 g, 5 mmol) in ethanol (15 mL) was heated at reflux for 12 h after the addition of N-ethyldiisoproylamine (2.45 mL, 15 mmol). After evaporation, the residue was chromatographed on a column (4.5 cm ID x 10 cm L) of silica gel by the use of chloroform - methanol system to give a glass of the acid (1.9945 g, 80% yield), ^{1}H-n.m.r. (chloroform-d - TMS): δ 2.56 (2H, t, J 7.6 Hz, C-C$\underline{H}_2$), 2.83 (2H, t, J 7.6 Hz, C-C$\underline{H}_2$), 3.01 (2H, t, J 6.8 Hz, C-C$\underline{H}_2$), 3.32 (2H, t, J 6.8 Hz, C-C$\underline{H}_2$), 3.64 (3H, s, OC$\underline{H}_3$), 6.75 (2H, d, J 8.8 Hz, Ph proton x 2), 6.98 - 7.45 (16H, m, Ph proton x 16).

Anal. Calcd for $C_{31}H_{30}O_4S$: C, 74.67; H, 6.06; S, 6.43. Found: C, 74.76; H, 6.15; S, 6.40.

A cellulose acetate derivative functionalized with 4-(2-hydroxyethylsulfonyl)dihydrocinnamate (**1**): A solution of 4-(2-monomethoxytrityloxyethylthio)dihydrocinnamic acid (2.7924 g, 5.6 mmol) and a cellulose acetate (D.S. = 1.77)[10](1.1794 g) in pyridine (28 mL) was, after the removal of moisture through azeotropic distillation from pyridine (5 mL) three times in vacuo, treated in the presence of 2,4,6triisopropylbenzenesulfonyl chloride (TPS; 4.0231 g, 14 mmol) and 1-methylimidazole (1-Meim; 2.24 mL, 28 mmol) at room temperature for 2 h. The resulting mixture was quenched with chilled water (5 mL) under stirring for 30 min. The mixture was extracted with chloroform (100 mL x 2) and the solvent was evaporated after washing with water (50 mL). The residue was dried through the azeotropic distillation from pyridine (5 mL) three times in vacuo, and was dissolved in pyridine (14 mL) to treat with acetic anhydride (4.7 mL) at room temperature for 5 h with stirring. The resulting solution was concentrated in vacuo and evaporation of the toluene (a small volume for each operation) solution of the residue was repeated until no smell of pyridine was detected. The residue thus obtained was dissolved in a mixture of 1,4-dioxane (130 mL) - acetic acid (9 mL), to which was added a solution of sodium tungstenate monohydrate (0.1868 g) in water (19 mL), and 30% aqueous hydrogen peroxide solution (28 mL) dropwise at 80°C taking 1 h, followed by further stirring for 1 h. After cooling down to room temperature, the mixture was extracted with chloroform (150 mL) - pyridine (20 mL), and the organic layer was washed with water (100 mL). After evaporation of the organic layer, the residue was dissolved in chloroform (30 mL), and the solution was added dropwise into ethanol (500 mL) with vigorous stirring. The resultant precipitates was gathered by filtration, and washed with ethanol (200 mL) to give monomethoxytrityl ether of **1** (2.9573 g) as a white powder after drying over anhydrous magnesium sulfate in vacuo. The white powder (2.6316 g) thus obtained was dissolved into a solution of 0.7 M zinc bromide in 7:3 chloroform - methanol[11] (30 mL), and the solution was stirred at room temperature for 2 h. The resulting solution was, after concentrating in vacuo to a volume of ca. 1/2, added dropwise into ethanol (400 mL). The precipitates thus obtained were gathered by filtration, and were washed with ethanol (100 mL) to give a white powder of **1** (1.8160 g) after drying over anhydrous magnesium sulfate in vacuo. The loaded amount of 4-(2-hydroxyethylsulfonyl)dihydrocinnamate function was estimated 1.65 mmol/g on the basis of analytical data for sulfur content, i.e., 5.30%, 1.65 mmol S/g.

Triethylammonium 5'-O-dimethoxytrityl-2'-O-(tetrahydropyran-2-yl)ribonucleosides 3'-(2-chlorophenyl)phosphates (**2**) and triethylammonium 5'-O-dimethoxytrityl-2'-deoxyribonucleosides 3'-(2-chlorophenyl)phosphates(**3**): N^3-Anisoyl-2'-O-(tetrahydropyran-2-yl)uridine,[17] N^4-anisoyl-2'-O-(tetrahydropyran-2-yl)cytidine,[17] N^6-benzoyl-2'-O-(tetrahydropyran-2-yl)-adenosine,[17] O^6-diphenylcarbamoyl-N^2-isobutyryl-2'-O-(tetrahydropyran-2-yl)guanosine,[17] N^3-benzoylthymidine,[18] N^4-anisoyl-2'-deoxycytidine,[19,20] N^6-(N,N-dimethylacetamidine)-2'-deoxyadenosine,[21] and O^6-diphenylcarbamoyl-N^2-isobutyryl-2'-deoxyguanosine[22] were subjected to 3'-(2-chlorophenyl)phosphorylation[23] after introducing dimethoxytrityl protecting group[24] at their 5'-position to give the corresponding **2** and **3**.

Syntheses of Diribonucleotides 3'-Phosphodiester Derivatives (**10**) by the Use of **1** as the Polymer-support; 1) Compound **10** (B_1 = U^{An}, B_2 = U^{An}): A solution of **1** (0.2424 g, 0.4 mmol) and **2** (B = U^{An}; 0.3170 g, 0.3 mmol) in pyridine (3 mL) was concentrated in vacuo,

and this operation was repeated twice after the addition of pyridine (3 mL), respectively. The residue thus obtained was dissolved in pyridine (3 mL), to which was added TPS (0.2726 g, 0.9 mmol) and 1-Meim (0.14 mL, 1.8 mmol), and the mixture was stirred for 1 h at room temperature. The resulting mixture was quenched with chilled water (0.5 mL) with stirring for 30 min, and the solution was extracted with methylene chloride (25 mL x 2). The organic solution was evaporated after washing with water (30 mL). Moisture involved in the residue was azeotropically removed by distillation from pyridine (3 mL x 3) in the manner as described in the first place. The residue thus obtained was treated with acetic anhydride (2.5 mL) in pyridine (7.5 mL) at room temperature for 5 h, and the resulting mixture was repeatedly distilled from toluene (a small volume) until no smell of pyridine was detected. A trace amount of the residue was taken out, and was dissolved in methylene chloride. The solution was poured into ethanol to give a powder of the cellulose acetate derivative bearing the unit of **2** (3.1 mg). The derivative was then treated with 2% solution of $TsOH \cdot H_2O$ in 7:3 chloroform - methanol (1 mL), and the color arising from the monomethoxytrityl function was developed by treating with a 3:2 mixture of 60% perchloric acid - ethanol. The resulting solution was subjected to determination of the amount of **2** loaded on **1** in the manner reported by Gait et al.[24] [λ_{max} 498 nm (ε 72,000)] and the amount was found to be 0.56 mmol/g, or 99% yield for the loading procedure. All the above residue was dissolved in 7:3 chloroform - methanol (10 mL) and the solution was treated with a solution of $TsOH \cdot H_2O$ (0.3954 g) in 7:3 chloroform - methanol (5 mL) at 0°C for 15 min with stirring. The resulting solution was, after neutralizing by the addition of 5% aqueous sodium hydrogencarbonate solution, extracted with chloroform, and the organic solution was concentrated in vacuo. The residue was poured into ethanol (200 mL) with vigorous stirring, after dissolving into chloroform (10 mL). The precipitates thus obtained was gathered by filtration to give a powder of **4** (0.3330 g, 0.674 mmol, 75% yield)(Step 1 up to here).

Subsequently, a solution of **2** (B = U^{An}; 0.3330 g) and **4** (0.2958 g, 0.28 mmol) in pyridine (3 mL) was concentrated in vacuo and this procedure was then repeated twice to remove moisture after the addition of pyridine (3 mL x 2), respectively. A solution of the residue in pyridine (2.8 mL) was treated with TPS (0.2544 g, 0.84 mmol) and 1-Meim (0.13 mL, 0.168 mmol) at room temperature for 1 h with stirring. The resulting solution was quenched by stirring with chilled water (0.5 mL) for 30 min, and extracted with methylene chloride (25 mL x 2). The extracts were combined to concentrate in vacuo after washing with water (20 mL). A trace amount of this residue was dissolved into methylene chloride and added dropwise into ethanol similarly as has been described in Step 1. The precipitates thus obtained were gathered by filtration to give a powder (2.9 mg), which was then subjected to the DMTr-determination in the same way as described in Step 1. The amount of loaded diribonucleotide was thus found to be 0.401 mmol/g, or 97% yield for the second loading procedure (Step 2 up to here).

All the residue was treated with 1:3 mixture of triethylamine - pyridine (8 mL) at room temperature for 2 h with stirring, and the resulting solution was concentrated in vacuo. The residue was dissolved in pyridine (10 mL), and the solution was added dropwise into ethanol (200 mL) with vigorous stirring. The resulting precipitates of vinyl sulfone derivative (**9**) arising from β-elimination reaction was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by reversed-phase silica gel column (2.5 cm ID x 10 cm L) chromatography by the use of acetone (40 - 60%) - 0.05 M TEAB solution to give the title **10** (0.2926 g, 58% yield). Incidentally, 0.0526 g (0.05 mmol) of uncoupled **2** was recovered in this case.

Compound **10** (B_1 = B_2 = U^{An}): Rf 0.28 (Solvent D) and 0.65 (Solvent E); ^{1}H-n.m.r. (chloroform-d - TMS): δ 1.21 (9H, t, J 7.3 Hz, N-CH_2-CH_3 x 3), 1.27 - 1.75 (12H, m, C-CH_2-C x 6), 2.95 (6H, q, N-CH_2-Me x 3), 3.41 - 3.68 (6H, m, H-5', 5", and O-CH_2-C x 2), 3.796, 3.80, 3.83, and 3.85 (12H, s x 4, OCH_3 x 4), 4.16 - 5.75 (12H, m, H-5 x 2, H-2' x 2, H-3' x 2, H-4' x 2, 5', 5", and O-CH-O x 2), 6.05, 6.11, 6.15, and 6.27 [2H, d x 4 (probably arising from diastereomers of phosphoryl functions involved therein), $J_{1',2'}$ 6.4 Hz, 6.4 Hz, 7.1 Hz, and 7.1 Hz, H-1' x 2), and 6.83 - 7.92 (31H, m, Ph proton x 29 and H-6 x 2).

Similarly, **10** (B_1 = U^{An}, B_2 = A^{Bz}) and **10** (B_1 = B_2 = A^{Bz}) were synthesized and the conditions used as well as the results obtained are summarized in Entries 2 and 3 in addition to those with respect to **10** (B_1 = B_2 = U^{An}) in Table 1.

Compound **10** (B_1 = U^{An}, B_2 = A^{Bz}): Rf 0.30 (Solvent D), 0.67 (Solvent E); ^{1}H-n.m.r.(chloroform-d - TMS): δ 1.19 - 1.66 (12H, m, C-CH_2-C x 6), 1.22 (9H, t, J 7.3 Hz, N-CH_2-CH_3

x 3), 2.96 (6H, q, N-CH_2-Me x 3), 3.04 - 3.78 (6H, m, H-5', 5", and O-CH_2-C x 2), 3.75 (6H, s, O-CH_3 x 2), 3.83 (3H, s, O-CH_3), 4.28 - 5.84 (11H, m, H-5, H-2' x 2, H-3' x 2, H-4' x 2, H-5', 5" and O-CH-O x 2), 6.15, 6.17, 6.34, 6.39 (2H, d x 4, $J_{1',2'}$ 7.1 Hz, 7.8 Hz, 8.1 Hz, and 7.3 Hz, respectively, H-1' x 2), 6.76 - 8.67 (33H, m, Ph proton x 30, H-2, 8 on adenyl, and H-6 of uracil moiety), and 9.28 (1H, br. s, N-H).

Compound **10** (B_1 = B_2 = A^{Bz}): Rf 0.29 and 0.32 (Solvent D), 0.66 and 0.69 (Solvent E); ^{1}H-n.m.r.(chloroform-d - TMS): δ 1.22 - 1.62 (12H, m, C-CH_2-CH_3 x 6), 1.25 (9H, t, J 7.3 Hz, N-CH_2-CH_3 x 3), 2.60 - 3.85 (6H, m, H-5', 5", and O-CH_2-C x 2), 3.00 (6H, q, N-CH_2-Me x 3), 3.74 and 3.75 (6H, s x 2, O-CH_3 x 2), 4.53 - 5.51 (10H, m, H-2' x 2, H-3' x 2, H-4' x 2, H-5', 5", and O-CH-O x 2), 6.26, 6.31, 6.36, and 6.39 (2H, d x 4, $J_{1',2'}$ 5.5 Hz, 5.9 Hz, 7.6 Hz, and 7.4 Hz, respectively, H-1' x 2), 6.74 - 8.79 (35H, m, Ph proton x 31, H-2 x 2, and H-8 x 2), and 9.20 - 9.44 (2H, m, N-H x 2).

Synthesis of Triethylammonium Tetraribonucleotide 3'-Phosphodiester Derivative (**11**; B_1 = B_2 = B_3 = B_4 = A^{Bz}): The title compound was synthesized by condensation of **5** (B_1 = B_2 = A^{Bz}) onto **1**, followed by dedimethoxytritylation, condensation with **5** (B_1 = B_2 = A^{Bz}), and, finally, its splitting-off from the polymer-support, in the similar manner as above described with respect to **10s**. The conditions used and the results are summarized in Table 2. The product gave Rf 0.10 (Solvent D), 0.45 (Solvent E); ^{1}H-n.m.r. (chloroform-d - TMS): δ 1.12 - 1.76 (24H, m, C-CH_2-C x 12), 1.23 (9H, t, J 7.3 Hz, N-CH_2-CH_3 x 3), 2.90 - 3.56 (8H, m, O-CH_2-C x 4), 2.97 (6H, q, N-CH_2-Me x 3), 3.68 - 3.92 (2H, m, H-5' and 5"), 3.72 (6H, s, O-CH_2 x 2), 4.12 - 5.72 (22H, m, H-2' x 4, H-3' x 4, H-4' x 4, H-5' x 3, H-5" x 3, and O-CH-O x 4), 6.10 - 6.35 (4H, m, H-1' x 4), 6.73 - 8.81 (57H, m, Ph proton x 49, H-2 x 4, and H-8 x 4), and 9.20 - 9.76 (4H, m, N-H x 4).

Synthesis of Triethylammonium Hexaribonucleotide 3'-Phosphodiester Derivative (**12**; B_1 = U^{An}, B_2 = A^{Bz}, B_3 = B_4 = U^{An}, and B_5 = B_6 = A^{Bz}): The title compound was synthesized by condensation of **10** (B_1 = U^{An}, B_2 = A^{Bz}) onto **1**, followed by dedimethoxytritylation, condensation with **10** (B_1 = B_2 = U^{An}), dedimethoxytritylation, condensation with **10** (B_1 = B_2 = A^{Bz}), and, finally, its splitting-off from the polymer-support, in the similar manner as above described with respect to **10s**. The conditions used and the results are summarized in Table 3. The product gave Rf 0.05 (Solvent D) and 0.27 (Solvent E).

Synthesis of the Undecaribonucleotide AAAAAAUUAUG (**20**): 1)(3-Carboxy)propionylation[26] of 5'-O-dimethoxytrityl-O^6-diphenylcarbamoyl-N^2-isobutyryl-2'-O-(tetrahydropyran-2-yl)-guanosine: A solution of the guanosine derivative (1.6670 g, 1.78 mmol) in methylene chloride (8.9 mL) was treated with succinic anhydride (0.3568 g, 3.57 mmol) and 4-dimethylaminopyridine (DMAP; 0.4362 g, 3.57 mmol) at room temperature for 30 min with stirring. The resulting mixture was quenched by treating with chilled water (5 mL) at room temperature for 30 min with stirring, and extracted with methylene chloride (25 mL x 2). The extracts were combined to wash with 0.1 M aqueous TEAB solution (20 mL x 3). After drying over anhydrous magnesium sulfate, the organic layer was concentrated in vacuo. The residue was purified by chromatography on a column (2.5 cm ID x 10 cm L) of silica gel by the use of methanol - methylene chloride system, to give a glass of 3'-O-[3-(carboxy)propionyl]-5'-O-dimethoxytrityl-O^6-diphenylcarbamoyl-N^2-isobutyryl-2'-O-(tetrahydropyran-2-yl)guanosine (1.7370 g, 94% yield): Rf 0.42 (Solvent A); ^{1}H-n.m.r.(chloroform-d - TMS): δ 1.12 - 1.19 [6H, m, C(CH_3)$_2$], 1.24 - 1.70 (6H, m, C-CH_2-C x 3), 2.51 - 2.84 (3H, m, CHMe$_2$ and CH_2-CO_2H), 2.96 - 3.23 (2H, m, O-CH_2-C), 3.29 - 3.49 (2H, m, CH_2-CO_2-), 3.67 - 3.82 (2H, m, H-5' and 5"), 3.74 and 3.79 (6H, s x 2, OCH_3 x 2), 4.30 - 4.35 (1H, m, H-4'), 4.68 - 4.74 (1H, m, O-CH-O), 5.25 (1H, dd, $J_{2',3'}$ 4.88 Hz, H-2'), 5.50 - 5.56 (1H, m, H-3'), 6.27 (1H, d, $J_{1',2'}$ 7.81 Hz, H-1'), 6.77 - 7.76 (23H, m, Ph proton x 23), 8.18 (1H, s, H-8), 8.31 (1H, s, N^2-H), and 8.54 - 8.62 (1H, m, -CO_2H).

Anal. Calcd for C, 65.01; H, 5.74; N, 7.98. Found: C, 64.74; H, 5.62; N, 7.92.

2)Loading of the Guanosine 3'-O-(3-Carboxy)propionyl Function with a Free Hydroxyl Group at 5'-Position onto **1**: A mixture of **1** (0.3636 g, 0.6 mmol) and the 3'-(3-carboxy)propionate (0.4140 g, 0.4 mmol) was azeotropically demoistured by distilling from pyridine (5 mL x 3) three times. A solution of the residue in pyridine (6 mL) was treated with TPS (0.3634 g, 1.2 mmol) and 1-Meim (0.20 mL, 2.4 mmol) at room temperature for 2 h with stirring. After quenching the mixture with chilled water (2 mL) at room temperature for 30 min with stirring, it was extracted with methylene chloride (10 mL x 3), the organic solution was

washed with water (20 mL), and, then, concentrated *in vacuo*; the residue was similarly subjected to azeotropic demoisture procedure using pyridine (5 mL x 3). It was then treated with 1:3 acetic anhydride - pyridine (15 mL) at room temperature for 5 h with stirring. The solution was concentrated *in vacuo*, and the residue was distilled from a small volume of toluene repeatedly until no smell of pyridine was detected. A trace amount of the residue was dissolved in methylene chloride, and the solution was added dropwise into excess amount of ethanol with vigorous stirring, to give a white powder of the cellulose acetate derivative bearing the objective guanosine function (4.3 mg). The product was then subjected to the DMTr-determination[25], and was found to load the function 0.5 mmol/g, or 98% yield for this loading procedure. A solution of all the residue in 7:3 chloroform - methanol was treated with TsOH·H_2O (0.3954 g) dissolved in 7:3 chloroform - methanol (5 mL) at 0°C for 15 min with stirring. The mixture was extracted with chloroform (50, 30, and then 20 mL), after neutralizing it with 5% aqueous sodium hydrogen carbonate solution, and the organic layer was concentrated *in vacuo*. The residue was dissolved in chloroform (10 mL), and was added dropwise into ethanol (200 mL) with vigorous stirring. The resulting precipitates were gathered by filtration to give the title polymer-support bearing the guanosine function with free 5'-hydroxyl group (0.4511 g, 66% yield, 0.589 mmol/g).

3)Successive Condensations of the Hexamer (**12**) and the Tetramer (**11**) Units onto the Cellulose Derivative Obtained in 2): The cellulose derivative (0.4244 g, 0.25 mmol) and **12** (0.4208 g, 0.1 mmol) was azeotropically demoistured by distilling from pyridine (5 mL x 3) three times, and the residue was dissolved in pyridine (5 mL). The solution was treated with TPS (0.1211 g, 0.4 mmol) and 1-Meim (0.065 mL, 0.8 mmol) at room temperature for 2 h with stirring. The resulting mixture was extracted with methylene chloride (20 mL x 3), after quenching it with chilled water (2 mL) at room temperature for 30 min, and the organic solution was washed with water (20 mL). After concentration of the organic solution *in vacuo*, the residue was again demoistured similarly as above described with pyridine (5 mL x 3). The dried residue was treated with 1:3 acetic anhydride - pyridine (10 mL) at room temperature for 5 h with stirring. The resulting mixture was concentrated *in vacuo*, and the residue was repeatedly dissolved in a small volume of toluene and concentrated until no smell of pyridine was detected. Yield of this loading procedure was determined similarly by the method reported by Gait *et al.*[25] to be 98%, or 0.117 mmol/g. A solution of all the residue in 7:3 chloroform - methanol (10 mL) was worked up similarly as above by the use of TsOH·H_2O (0.3945 g) in 7;3 chloroform - methanol (5 mL)[dedimethoxytritylation; Yield of the cellulose acetate derivative bearing the corresponding heptamer was 87% (0.7210 g, 0.121 mmol/g)]. The yield of the subsequent introduction of **11** (0.6424 g, 0.219 mmol) onto **17** (0.087 mmol) by the use of TPS (0.1990 g, 0.657 mmol) and 1-Meim (0.107 mL, 1.314 mmol) in the similar manner, giving the cellulose derivative bearing the objective undecamer (**18**), was 79% (0.7911 g, 0.087 mmol/g). The overall yield was thus 69%. Incidentally, was recovered the tetramer **11** (0.2180 g, 0.074 mmol) in this case.

4)Splitting-off and Purification of **20**: The cellulose derivative **18** (44.8 mg, 3.97 mol) was dissolved in a solution of 0.5 M N^1,N^1,N^3,N^3-tetramethylguanidium (Z)-2-pyridinealdoximate (TMG·PAO) in 9:1 pyridine - water (0.6 mL), and the solution was allowed to stand at room temperature for 1 day. To the resulting mixture, was added ethanol (30 mL), and the vinyl sulfone derivative of cellulose acetate precipitated out was removed by centrifugation (3,000 r/m) at 4°C for 10 min. The supernatant was concentrated *in vacuo*, and the residue was dissolved in 28% aqueous ammoniacal solution (20 mL), which was then allowed to stand at 55°C for 6 h. The residue obtained by concentration *in vacuo* was dissolved in aqueous 50 mM TEAB solution, 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 50mM TEAB solution]. Elution of the column was performed successively with 18% acetonitrile - aqueous 50 mM TEAB (10 mL) for the removal of TMG·PAO etc., and with 35% acetonitrile - aqueous 50 mM TEAB solution (10 mL), gave a fraction containing the objective undecamer (**19**), whose 5'-terminus and all the 2'-hydroxyl groups were protected with DMTr and THP groups, respectively. This fraction was then lyophilized, and the residue was dissolved in water (1 mL). One of its 10 aliquots was subjected to purification through HPLC with 10 - 50% acetonitrile - aqueous 0.1 M TEAA solution to give remarkably simplified HPL chromatogram as shown in Fig. 1. The fraction corresponding to the central portion of the main peak was concentrated *in vacuo* to dryness, and the residue was dissolved in water (1 mL). The aqueous solution was concentrated *in va-*

cuo to dryness and the residue was kept under diminished pressure until no smell of triethylamine was detected. The resulting residue was dissolved in hydrochloric acid (pH 2.0; 5 mL), and the solution was allowed to stand at room temperature for 2 days. The solution was neutralized with diluted aqueous ammoniacal solution, and washed with ethyl acetate (10 mL x 3). The aqueous layer was concentrated to dryness. The residue was dissolved in water (200 μL), and 180 μL of which was then subjected to purification through HPLC with 5 - 19% acetonitrile - aqueous 0.1 M TEAA solution to give the product giving the HPL chromatogram as shown in Fig. 2-I. The fraction corresponding to the central portion of the HPL chromatogram was separated and lyophilized to give a pure sample of the completely deprotected undecamer (1.51 A_{260} units), whose HPL chromatogram is shown in Fig. 2-II. Furthermore, **20** gave a very clear band in electrophoresis on a 20% polyacrylamide gel containing 7 M urea after 5'-O-[^{32}P]-phosphorylation in terms of [γ-^{32}P]-ATP and T4 polynucleotide kinase according to the method reported.[15] The result obtained by the electrophoresis is shown in Fig. 3; the reference compounds of 4mer and 18mer for the electrophoresis were synthesized through the orginary solid-phase procedure by the use of Zeon H-6 DNA Synthesizer.[27]

5)Structure Confirmation of **20**: To a solution of **20** (0.1 A_{260} unit) in water (86.32 μL), were added an aqueous 1 M ammonium acetate buffer solution (pH 5.3; 10 μL) and a solution of nuclease P1 (Yamasa Shoyu Co., Ltd.) in the same buffer solution (3 μL of a solution prepared by dissolving 1 mg of the enzyme in 5 μL of water), and the resulting solution was allowed to stand at 37°C for 30 min. The mixture of the products thus obtained were analyzed through HPLC with 2 - 25% acetonitrile - aqueous 0.05 M ammonium dihydrogenphosphate solution (pH 5.2) to give an HPL chromatogram as shown in Fig. 4.

Syntheses of Triethylammonium Di-2'-deoxyribonucleotides 3'-Phosphodiester Derivatives (**13**) by the Use of **1** as the Polymer-support: Similar syntheses as above described gave diastereoisomeric mixtures of **13**, and the conditions used as well as the results obtained are summarized in Table 4.

Compound **13** (B_1 = B_2 = T^{Bz}): Rf 0.29 (Solvent D); ^{1}H-n.m.r.(chloroform-d - TMS): δ 1.24 (9H, t, J 7.31 Hz, N-CH_2CH_3 x 3), 1.27 (3H, s, CH_3), 1.35 (3H, s, CH_3), 2.05 - 2.70 (4H, m, H-2' x 2 and H-2" x 2), 2.97 (6H, q, N-CH_2-CH_3 x 3), 3.78 (6H, s, OCH_3 x 2), 3.30 - 4.50 (6H, m, H-4' x 2, H-5' x 2, and H-5" x 2), 4.95 - 5.40 (2H, m, H-3' x 2), 6.29 - 6.45 (2H, m, H-1' x 2), and 6.30 - 8.06 (33H, m, Ph proton x 31 and H-6 x 2).

Compound **13** (B_1 = B_2 = $^{DPC}G^{iBu}$): Rf 0.17 (Solvent D); ^{1}H-n.m.r.(chloroform-d - TMS): δ 1.24 [21H, m, $C(CH_3)_2$ x 2 and N-CH_2-CH_3 x 3], 1.27 (14H, m, $CHMe_2$ x 2, H-2', 2", N-CH_2-Me x 2, H-5' x 2, and H-5" x 2), 3.70 (6H, s, OCH_3 x 3), 4.50 - 4.65 (2H, m, H-4' x 2), 5.30 - 5.55 (2H, m, H-3' x 2), 6.30 - 6.45 (2H, m, H-1' x 2), 6.66 - 7.64 (41H, m, Ph proton x 41), 8.05 - 8.07 (2H, s, H-8 x 2), 8.55 (1H, s, NHCO), and 8.80 (1H, s, NHCO).

Compound **13** (B_1 = B_2 = A^{aca}): Rf 0.34 (Solvent D); ^{1}H-n.m.r.(chloroform-d - TMS): δ 1.27 (9H, t, 7.3 Hz, N-CH_2-CH_3 x 3), 2.1 [6H, s, $C(CH_3)NMe_2$ x 2], 2.85 - 2.90 (4H, m, H-2' x 2 and 2" x 2), 2.90 (6H, q, N-CH_2-Me x 3), 3.13 [12H, m, $N(CH_3)_2$ x 2), 3.34 (4H, m, H-5' x 2 and 5" x 2), 3.72 (6H, s, OCH_3 x 2), 4.40 (2H, m, H-4' x 2), 5.40 - 5.55 (2H, m, H-3' x 2), 6.43 (2H, m, H-1' x 2), 6.70 - 7.50 (21H, m, Ph proton x 21), 8.0 (2H, s, H-2 x 2), and 8.55 (2H, s, H-8 x 2).

Synthesis of Triethylammonium Tri-2'-deoxyribonucleotide 3'-Phosphodiester Derivative (**14**; B_1 = C^{An}, B_2 = B_3 = T^{Bz}): The title trinucleotide was synthesized by the coupling reaction of **2** (B_1 = C^{An}) loaded on **1** with **13** (B_1 = B_2 = T^{Bz}) similarly as described in the synthesis of **10**. The conditions used and the result obtained are summarized in Table 5.

Synthesis of Triethylammonium Tetra-2'-deoxyribonucleotide 3'-Phosphodiester Derivative (**15**; B_1 = B_2 = A^{aca}, B_3 = B_4 = $^{DPC}G^{iBu}$): The title tetranucleotide was synthesized by the coupling reaction of **13** (B_1 = B_2 = A^{aca}) loaded on **1** with **13** (B_1 = B_2 = $^{DPC}G^{iBu}$) similarly as described in the synthesis of **10**. The conditions used and the result obtained are summarized in Table 6.

Synthesis of Octa-2'-deoxyribonucleotide dGGAATTCC (**25**): 1)3'-O-(Carboxy)propionylation[26] of N^4-Anisoyl-2'-deoxy-5'-O-dimethoxytritylcytidine; The reaction was performed similarly as described in the synthesis of **20**. The resulting 3'-(3-Carboxy)propionate gave ^{1}H-n.m.r.(chloroform-d - TMS): δ 2.25 - 2.70 (6H, m, H-2', 2", and CO-CH_2-CH_2-CO), 3.40 (2H, m, H-5' and 5"), 3.78 (6H, s, OCH_3 x 2), 3.83 (3H, s, OCH_3), 4.22 (1H, m, H-4'),

5.41 (1H, m, H-3'), 6.25 (1H, t, $J_{1',2'}$ 6.35 Hz, H-1'), 6.75 - 7.39 (16H, m, Ph proton x 15 and H-5), 7.95 (2H, d, J 10.83 Hz, o-Ph proton x 2), and 8.11 (1H, d, $J_{5,6}$ 8.49 Hz, H-6).

2)Loading of the 2'-Deoxycytidine 3'-O-(3-Carboxy)propionyl Function with a Free Hydroxyl Group at 5'-Position onto **1**: To a solution of the 3'-[3-(carboxy)propionate] (0.802 g, 1.05 mmol) and **1** (1.63 mmol S/g; 0.825 g, 1.34 mmol) in pyridine (40 mL), were added TPS (0.954 g, 3.15 mmol) and 1-Meim (0.50 mL, 6.3 mmol), and the resulting mixture was stirred at room temperature for 4 h. A cellulose polymer-support loading the title function **21** (1.319 g; 0.665 mmol S/g) was obtained through quenching the reaction by the addition of chilled water (2 mL), followed by the work-up similar to that described above in 2) of the synthesis of **20**.

3)Successive Condensations of the Trimer and Tetramer Units onto the Cellulose Derivative Obtained in 2): To a solution of **14** (B_1 = C^{An}, B_2 = B_3 = T^{Bz}; 0.267 g, 0.132 mmol) and (0.665 mmol S/g; 0.461 g, 0.307 mmol) in pyridine (20 mL), were added TPS (0.1196 g, 0.395 mmol) and 1-Meim (0.063 mL, 0.79 mmol), and the resulting mixture was stirred at room temperature for 2 h. Quenching the reaction with chilled water, followed by the work-up similar to that described in the undecaribonucleotide synthesis, gave the cellulose derivative loading the tetramer unit with a free hydroxyl group at the 5'-terminus **22** (B_1 = B_2 = C^{An}, B_3 = B_4 = T^{Bz}; 0.6685 g, 0.171 mmol S/g, 86% yield). Compound **22** (0.171 mmol S/g; 0.4435 g, 0.076 mmol) was then subjected to the coupling reaction with **15** (B_1 = B_2 = A^{aca}, B_3 = B_4 = $^{DPC}G^{iBu}$; 0.432 g, 0.154 mmol) in pyridine (20 mL) by the use of TPS (0.133 g, 0.44 mmol) and 1-Meim (0.07 mL, 0.88 mmol) at room temperature for 2 h, followed by quenching with chilled water (2 mL) and the work-up described in the undecaribonucleotide synthesis, to give a cellulose derivative loading d[GGAATTCC] unit **23** (0.612 g, 0.113 mmol S/g, 91% yield).

4)Splitting-off, Purification, and Structure Confirmation of the Octa-2'-deoxyribonucleotide, dGGAATTCC (**25**): Compound **23** (0.030 g, 3.39 μmol) was treated with a solution of 0.5 M TMG·PAO in 9:1 pyridine - water (0.6 mL) at room temperature for 1 day, and the resulting cellulose vinyl sulfone derivative was removed by pouring the reaction mixture into ethanol (30 mL) with vigorous stirring, followed by filtration. The filtrate was concentrated in vacuo to dryness, and the residue was then treated with conc. aqueous ammonical solution (20 mL) at 55°C for 12 h. Purification of the resulting DMTr-dGGAATTCC (**24**) successively through SEP-PAK and HPLC afforded an HPL chromatogram proving its predominant formation (See Fig. 5). Half volume of the fraction corresponding to the main peak was subjected to dedimethoxytritylation by treating with 80% aqueous acetic acid solution (1.31 mL) at room temperature for 30 min. The resulting mixture was, after concentration in vacuo to dryness, dissolved in water, and washed with diethyl ether. The aqueous layer was concentrated in vacuo to dryness, and the residue was dissolved in water (1 mL). The aqueous solution (300 μL) was subjected to purification through HPLC to give dGGAATTCC **25** (1.95 A_{260} units); its HPL chromatogram is shown in Fig. 6. Structure confirmation of **25** (0.3 A_{260} unit) was performed by degradation with snake venom phosphodiesterase (Beohringer Mannheim; 10 μL of the solution prepared by dissolution of 1 mg of the enzyme in 0.5 mL of water) at 37°C for 2 h. and then with alkaline phsphatase (Beohringer Mannheim; 10 μL of the solution prepared by dissolution of 10 mg of the enzyme in 1 mL of water) at 25°C for 1 h. The resulting solution was analyzed by HPLC as usual to give reasonable proportion of each 2'-deoxyribonucleoside unit; the HPL chromatogram is shown in Fig. 7.

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