Solid Phase Synthesis of Oligodeoxyribonucleotides Utilizing the Phenylthio Group as a Phosphate Protecting Group

Jun-ichi MATSUZAKI, Kyoko KOHNO, Shin-ichiro TAHARA, Mitsuo SEKINE, and Tsujiaki HATA*
Department of Life Chemistry, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227
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Oligodeoxyribonucleotide synthesis utilizing the phenylthio group as a phosphate protecting group was applied to the solid phase method. The base residues of deoxyguanosine and deoxyadenosine were protected with bis(isobutyryloxy)ethylene (Bibe) and phthaloyl groups to avoid the base modification and depurination, respectively. A key synthetic intermediate of N^2-isobutyryl-N^1,N^2-bis(isobutyryloxy)ethylenedeoxyguanosine was prepared in high yield by four-step reaction from deoxyguanosine and used for preparation of the building blocks of deoxyguanosine required for the polymer support synthesis. Two kinds of polymer supports, i.e., 1% cross-linked polystyrene and controlled pore glass were chosen. The latter was employed for the synthesis of dodecadeoxyribonucleotides by using an automated DNA synthesizer.

In chemical synthesis of oligodeoxyribonucleotides, application of the phosphotriester[1] and phosphoramidite[2] methods to the solid phase synthesis has enabled us to prepare rapidly and practically DNA fragments of defined sequences.[3] Although the latter approach became accessible more successfully to molecular biologists, the former has recently been remarkably improved by introduction of new condensing agents and protecting groups.[4] Especially, we have also developed our own triester approach utilizing the phenylthio group as a phosphate protecting group.[5] In this paper, we wish to report application of our approach to the solid phase method.

Results and Discussion

Partially protected nucleosides (**1a—e**) were prepared by the literature method.[6,7] The amino goup of deoxyadenosine was protected with the benzoyl or phthaloyl group. Our recent study showed that the latter protecting group had considerable retarding effects on the depurination.[7]

Reese[8] and we[9] originally reported side reactions at the O^6-position of guanine residues when the remaining reactive site was not protected during condensations. To avoid such undesired reactions a number of new protecting groups have been proposed showing remarkable improvements.[10-15] In a previous paper,[12] we demonstrated the utility of the bis(isobutyryloxy)ethylene (Bibe) group as the so-called protected protecting group for the guanine base. This protecting group was readily removed under the same conditions as used for removal of the usual *N*-acyl protecting groups. However, the Bibe group has been introduced into the guanine residue only at the last stage of the synthesis of the deoxyguanosine monomer unit.[11] This is a drawback of the Bibe group although its introduction to the guanine base could be very easily performed by simple addition of glyoxal followed by acylation without coloration. Therefore, we searched for synthetic routes to N^2-isobutyryl-N^1,N^2-bis(isobutyryloxy)ethylenedeoxyguanosine (**1f**) which was a versatile synthetic intermediate for synthesis of

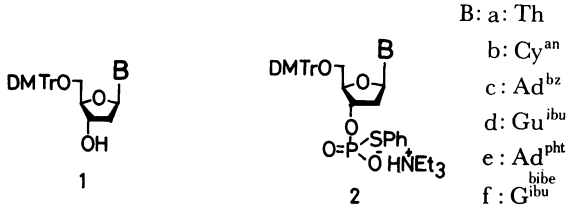


Fig. 1. Nucleoside and nucleotide units for the synthesis.

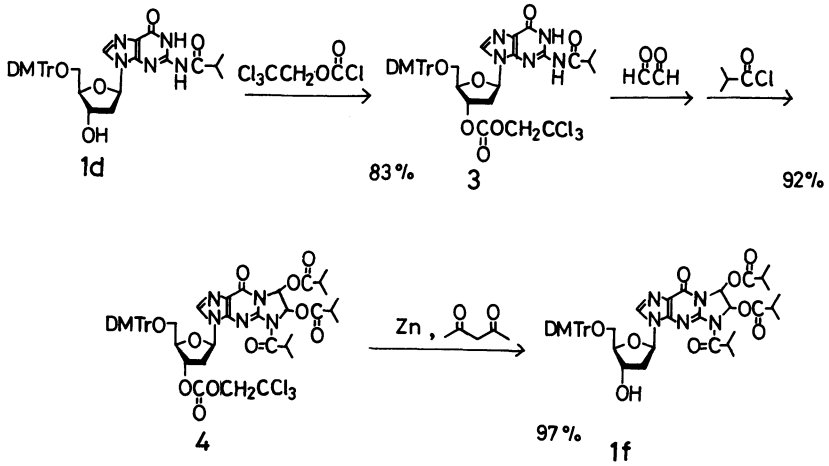


Fig. 2. Introduction of the bibe group into guanosine.

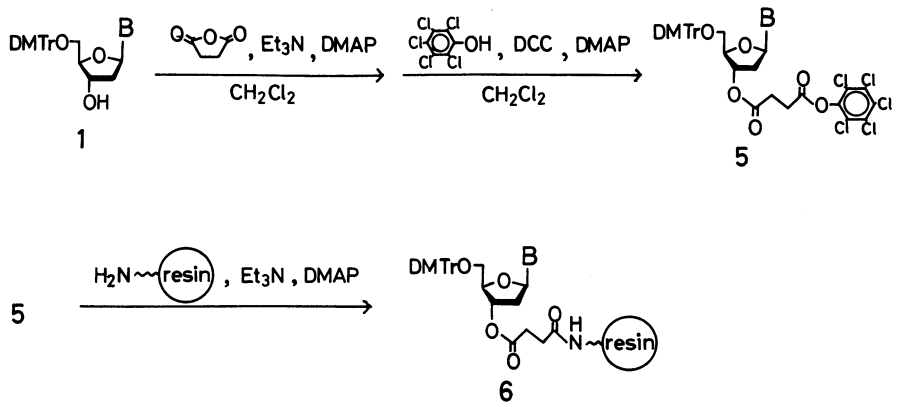


Fig. 3. Immobilization of the nucleosides onto resins.

appropriately protected deoxyguanosine derivatives (**5**, B=G^{ibu} and **2f**). For this purpose, the use of the transient protection procedure using trimethylsilyl chloride seemed to be difficult since the glyoxalation required a large excess of glyoxal which might work as a nucleophile toward the silyl group. Therefore, we chose a more stable protecting group as the 3′,5′-diol protecting group, i.e., the 2,2,2-trichloroethoxycarbonyl (TCECb)group.[7] As s hown in Fig. 2, deoxyguanosine was allowed to react with 2,2,2-trichloroethyl chloroformate and then the Bibe group was introduced to the guanine moiety. Finally, the fully protected nucleoside (**4**) was converted by treatment with zinc-acetylacetone to the desired compound (**1f**) in a 74% overall yield from **1d**.

Solid supports chosen in this study were controlled pore glass (CPG)[17] as an unswellable inorganic resin and 1% cross-linked polystyrene (P.S.)[18] as a swellable organic resin. The 3′-free nucleosides (**1**) were immobilized to the polymer supports according to the modified method of Itakura[19] (Fig. 3). Succinylation of **1** was performed by treatment with succinic anhydride in the presence of triethylamine and a catalytic amount of 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 followed by esterification of the resulting carboxylic acid with pentachlorophenol by dicyclohexylcarbodiimide (DCC) and DMAP in CH_2Cl_2 after extraction. The activated nucleosides (**5**) were immobilized to the 3-aminopropyl group on the resin. The amounts of the nucleosides loaded on the supports were determined by spectroscopic estimation of DMTrOH released by the acid treatment and summarized in Table 2.

Oligomer Synthesis on Polystyrene. One % cross-linked polystyrene has been known to be one of suitable supports for the synthesis of oligonucleotides as reported by Itakura[18a] and Ohtsuka.[18b] We chose this support to prepare oligothymidylates by the use of the phenylthio group and bifunctional condensing reagents.[20] The phosphodiester (**2a**) of thymidylic acid was condensed to the thymidine resin (**6a**) by isodurenedisulfonyl chloride (DDS)[4a] and 3-nitro-1,2,4-triazole (NT)[21] for 1 h. For the detritylation, 1% trifluoroacetic acid (TFA) was used and the capping reaction was performed in the usual way. Reasonable results were obtained (Table 3) but not yet satisfactory. To realize a more smooth reaction on the swellable support, solvents capable of increasing the swellability in the reaction should be chosen. Therefore, dichloromethane was used in the condensation reaction and a few drops of pyridine was added to dissolve the catalyst, NT. A pentathymidylate was synthesized by using this solvent system under the same conditions as used in the above experiment but the reaction required 30 min. Better yields were obtained and the first coupling was optimized (Table 4). The capping reaction was performed by the use of acetic anhydride-triethylamine in dichloromethane in the presence of 0.1 M† DMAP for 5 min.

Table 1. Yields of the Activated Ester (**5**)

B	Succinylation/h	Esterification/h	Yield/%
Th	3	2	81
Cy^{an}	4	1	92
Ad^{bz}	4	1	87
Gu^{ibu}	4	2	96
Gu^{bibe}_{ibu}	4	2	88

Deprotection of the fully protected pentathymidylate was performed in two ways: A) 1) 0.1 M NaOH, pyridine-H_2O (1 : 1, v/v), 3 h 2) 80% AcOH, 15 min. B) 1) 2-pyridinecarbaldehyde oxime-tetramethylguanidine (TMG), pyridine-H_2O (9 : 1, v/v), 22 h 2) 80% AcOH, 15 min.

Isolation of the oligomer was performed by ion exchange HPLC (Partisil 10 SAX) and the yields of $T(Tp)_3T$ were 22 and 43% for methods A and B, respectively. These results would be ascribed to the swellability of the resin in the solvent used in the deprotection (step 1). Even in step 1 of method A, the resin was less swelled than in the condensation reaction where CH_2Cl_2 was used. After removal of the oligomer by filtration and washing with pyridine-H_2O (9 : 1, v/v),

† 1 M=1 mol dm^{-3}.

Table 2. Immoblization of Nucleosides on Resins

1% Cross linked polystyrene		Controlled pore glass (mesh 120/200)		
B	Nucleoside	B	Pore size	Nucleoside
	μmol g^{-1}		A	mmol g^{-1}
Th	100	Cy^{an}	171	118
Cy^{an}	83	Th	569	24
Ad^{bz}	109	Cy^{an}	569	32
Gu^{bibe}_{ibu}	108	Cy^{an}	547[a)]	32
		Ad^{bz}	569	42
		Gu^{bibe}_{ibu}	569	27
		Cy^{an}	1489	24

a) Mesh 200/400 was used.

Table 3. Synthesis of Oligothymmidylate on Polystyrene in Pyridine

Condensation	7th	6th	5th	4th	3rd	2nd	1st
Yield/%	79	87	100	25	101	100	70
	43	53	60	86	77	91	73
	66	64	68	79	74	73	84
	80	83	86	84	94	97	66

Condensation: Tymidine P.S. (50 mg, 5 μmole), phosphodiester (T, 50 μmole), DDS (100 μmole), NT (200 μmole), pyridine (500 μl), r.t., 1 h. Capping: Acetic anhydride-pyridine (1:9, v/v, 1 ml), DMAP (0.1 mmole), r.t., 5 min.

Table 4. Synthesis of Oligothymidylate on Polystyrene in CH_2Cl_2

Condensation	4th	3rd	2nd	1st
Yield/%	91	91	89	92
	91	92	90	100

Condensation: Tymidine P.S. (50 mg, 5 μmole), phosphodiester (T, 50 μmole), DDS (100 μmole), NT (200 μmole), CH_2Cl_2-pyridine (50:3, v/v, 500 μl), r.t., 30 min. Capping; Acetic anhydride-triethylamine-CH_2Cl_2 (2:3:16, v/v/v, 1 ml), DMAP (0.1 mmole), r.t., 5 min.

TFA treatment of the resulting resin indicated that some amounts of oligomers remained in the cage of the resin. This phenomenon was not observed in the case of the CPG described below.

Oligomer Synthesis on the Glass Support. Controlled pore glass (CPG) has been reported as a suitable support for the synthesis of oligodeoxyribonucleotides in the phosphotriester[22)] and the phosphoramidite[17)] approaches. The thymidylic acid unit was condensed to deoxyribonucleosides on three kinds of CPG (Funakoshi) having the pore sizes of 170, 560, and 1500 A. The yields were essentially dependent on the pore size of resin and the reaction on 560 A CPG gave good results (Table 5). A similar result was obtained by Köster[17b)] in his eary study on polymer supported synthesis. In this study, a rapid method for condensation reported by Effimov[23)] was introduced to the present system by use of CPG. The sequence of dGpTpApC

Table 5. First Coupling Yields on CPGs

Pore size/A	Mesh	Coupling time/h	Yield/%
171	120—200	1	63
559	120—200	1	80
547	200—400	1	89
1489	120—200	1	11

Condensation: Cytidine CPG (100 mg, 3.2 μmole), phosphodiester (T, 32 μmole), DDS (64 μmole), NT (96 μmole), pyridine (320 μl), r.t., 1 h. Capping: The same conditions described as in Table 3.

was synthesized to search for a suitable condensation system. A phosphodiester (20 equiv), a condensing agent (20 equiv), and *N*-methylimidazole (MeIm) (30 equiv) were used in CH_3CN and the reaction was performed for 40 min. The bifunctional condensing reagent of DDS was not suitable because of its low solubilities compared with monofunctional reagents. A better result was obtained by using mesitylenesulfonyl chloride (MS) as the condensing reagent (Table 6). 2,4,6-Triisopropylbenzenesulfonyl chloride (TPS) gave relatively low yields. The yield, however, slightly increased with an increase of MeIm (100 equiv). Low viscosity of CH_3CN and rigidity of CPG were suitable for synthesis of oligomers by the use of a fully automated DNA synthesizer. To ascertain the coupling efficiency of the MS-MeIm system on CPG, we tried to prepare three kinds of fully protected sequences of dodecadeoxyribonucleotides involving $dT(pT)_{11}$ (**7**), dCpApTpTpApTpTpApApTpApC (**8**), and dGpTpApTpTpApApTpApApTpG (**9**). The latter two sequences were corresponding to a DNA analog of the 5′-terminus of brome mosaic virus (BMV) mRNA No. 4 filament[24)] and its complementary sequence (Fig. 4), respectively. In the case of **8**, both the stepwise condensation and the block condensation were tried. The elongation program of DNA synthesizer (SAM 1, Biosearch) is presented in Table 7 and the yields of the condensation reactions are summarized in Fig 5. Among the fully protected dodecadeoxyribonucleotides synthesized on CPG, the protected dodecamers **8b** and **9** were deprotected according to the following conditions: 1) 0.1 M NaOH, pyridine-H_2O

Table 6. Synthesis of dGTAC on CPG

Condensing reagent	Azole	Coupling time	Yield/%			
equiv	equiv	min	1st	2nd	3rd	Average
DDS (20)	NT (30)	60	85	68	76	44
MS (20)	MeIm (30)	30	70	96	98	88
TPS (20)	MeIm (30)	30	80	86	83	83
TPS (20)	MeIm	30	82	91	88	87

Condensation: Cytidine CPG (100 mg, 3.2 μmole), phosphodiester (32 μmole), condensing reagent (64 μmole), azole (96 mg, 320 μmole), CH_3CN (320 μl), r.t., 30 h. Capping: The same conditions described as in Table 4.

Table 7. Elongation Cycle of the Automated Synthesizer

Function	Reagent or/and solvent	Time
Wash	CH_2Cl_2	2:00
Deblock	1% TFA-CH_2Cl_2	1:30
Wash	CH_2Cl_2	3:00
Wash	CH_3CN	2:00
Wash	dry CH_3CN	2:00
Coupling	phosphodiester (10 eequiv)	
	MS (93 equiv)	
	MeIm (450 equiv)	40:00
Wash	CH_3CN	2:00
Capping	Ac_2O/DMAP/pyridine/THF	5:00
Wash	CH_3CN	3:00

m^7GpppGpUpApUpUpApApUpApApUpGp------ BMV mRNA No.4

$5'$GPTPAPTPTPAPAPTPAPAPTPG$3'$

$3'$CPAPTPAPAPTPTPAPTPTPAPC$_5'$

Fig. 4. DNA analogue of 5′-teminus of BMV mRNA No.4 and its complementary sequence.

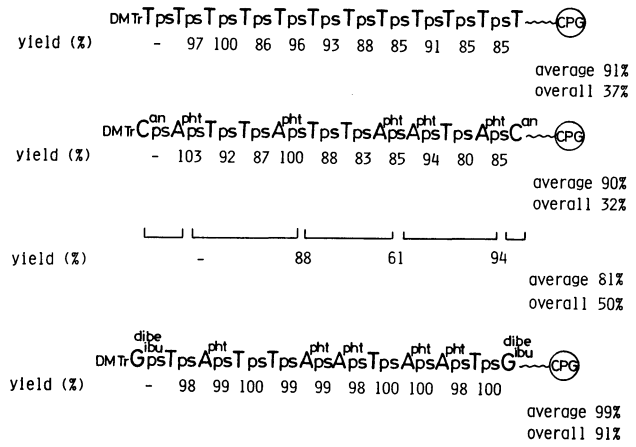


Fig. 5. Results of the automated synthesis of dodecamers.

(1 : 1, v/v), 0 °C, 1 h, 2) concd NH_4OH, r.t. for 30 h, 50 °C for 1 h, 3) 80% AcOH, r.t., 15 min.

Isolation of the unprotected oligomer **8b** and **9** was performed by reversed phase HPLC (μBondapak C_{18}) in the usual manner. A typical example is shown in Fig. 6a. This standard separation procedure gave finally the pure 12 mers **8b** and **9** in 18 and 4% yields. An alternative procedure for isolation of the 12 mer **9**

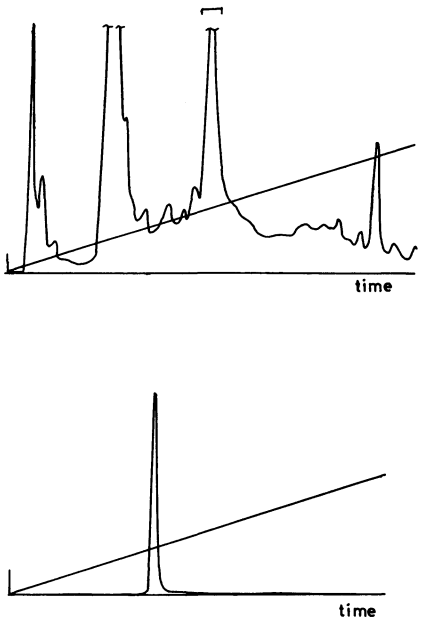
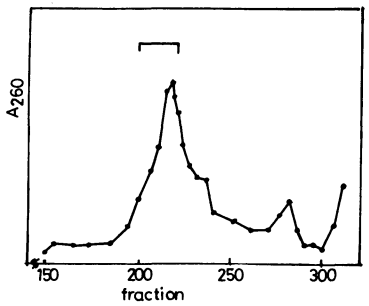


Fig. 6a. Chromatograms of dGTATTAATAATG on μBondapak C_{18}. Upper: After ammonia treatment, lower: Finally purified product.



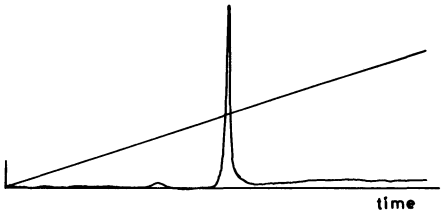


Fig. 6b. Chromatograms of dGTATTAATAATG on Sephadex G50 column (upper) and purified product on μBondapak C_{18} (lower).

was also conducted by using gel permeation column chromatography (Sephadex G 50). This gave an improved yield (12%) of the 12 mer. The separation profiles of the 12 mer are shown in Fig. 6b. The oligomers obtained were characterized by polyacrylamide gel electrophoresis (PAGE) and a modified Maxam-Gilbert sequencing method.[25]

Experimental

General Remarks. ^{1}H NMR spectra were recorded at 100 MHz on a JNM-PS-100 spectrometer using ppm according to tetramethylsilane as an internal reference. UV spectra were obtained on a Hitachi 124 spectrophotometer. Column chromatography was performed with silica gel C-200 purchased from Wako Co., Ltd. Thin-layer chromatography was performed on precoated TLC plates, silica gel 60 F-254 (Merck). Reagent grade pyridine was distilled twice from *p*-toluenesulfonyl chloride and CaH_2 and then stored over molecular sieves 4A. Dichloromethane was dried over phosphorus pentoxide overnight, decanted, distilled from potassium carbonate and then stored over molecular sieves 4A. Acetonitrile was distilled from calcium hydride and stored over molecular sieves 4A. 3-Aminopropyl polystyrene (1% cross linked) was purchased from Peptide Laboratory and aminopropyl CPGs from Electro Nucleonics Inc.. Deoxynucleosides were purchased from Yoshitomi-Seiyaku Co., and properly protected according to the literature.[4,5] The phosphodiester units were prepared according to our previous paper.[4] Ion-exchange HPLC was performed on a Partisil 10 SAX (Whatman) column using a linear gradient of 0.05 M KH_2PO_4 (pH 6.4), 20% CH_3CN to 0.5 M KH_2PO_4 (pH 6.5), 20% CH_3CN for 32 min at a flow rate of 1.5 ml ml^{-1}. Reversed phase HPLC was performed on a μBondapak C_{18} (Waters) using a liner gradient of 0.1 M NH_4OAc to 40% CH_3CN in the same buffer for 40 min at a flow rate of 2 ml at 50 °C. Elemental analysis was performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(2,2,2-trichloroethoxycarbonyl)-N^2-isobutyryldeoxyguanosine (3). 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-isobutyryldeoxyguanosine (3.2 g, 2 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and dissolved in dry pyridine (30 ml). 2,2,2-Trichloroethyl chloroformate (865 µl, 6 mmol) was added dropwise and the solution was stirred at room temperature for 2 h. Then the mixture was transferred into a separatory funnel with $CHCl_3$ and washed twice with 5% $NaHCO_3$. The aqueous layers were back-extracted with $CHCl_3$ and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the last traces of pyridine were coevaporated with toluene. The resulting foam was applied to a column of silica gel and eluted with CH_2Cl_2 containing methanol (0—1%, v/v) to give the title compound (3.24 g, 83%); mp 135 °C (decomp); ^{1}H NMR ($CDCl_3$) δ=0.98 (m, 12 H, $CH(CH_3)_2$), 2.69 (m, 2H, 2′H), 3.42 (m, 4H, 5′H, and $CH(CH_3)_2$), 3.80 (s, 6H, CH_3), 4.35 (m, 1H, 4′H), 4.79 (s, 2H, CH_2CCl_3), 5.58 (m, 1H, 3′H), 6.24 (m, 1H, 1′H), 6.82 (m, 4H, DMTr), 7.22—7.56 (m, 9H, ArH), 7.84 (s, 1H, 8).

Found: C, 55.62; H, 4.88; N, 8.33%. Calcd for $C_{38}H_{38}N_5O_9Cl_3$: C, 55.99; H, 4.70; N, 8.59%.

5′-*O*-(4,4′-Dimethoxytrityl)-N^2-isobutyryl-N^1,N^2-bis(isobutyryloxy)ethylenedoxyguanosine (4). Introduction of the Bibe group to **3** was performed according to our previous paper[6] in 92% yield. The fully protected guanosine derivative (2.2 g, 2 mmol) was coevaporated with dry pyridine (3×3 ml) and dissolved in dry pyridine (30 ml). To the solution, activated zinc powder (2.6 g, 40 mmol) and acetylacetone (4.1 ml, 40 mmol) was added and the suspension was stirred at room temperature. After 1 h, the solid material was filtered off and washed with pyridine. The filtrate and the washings were concentrated to one half the volume, transferred into a separatory funnel with $CHCl_3$, and washed three times with phosphate buffer (pH 6.0). The aqueous layers were back-extracted with $CHCl_3$ and the organic layer was washed with water. The organic layers were combined and dried over Na_2SO_4 and the solvent was removed under reduced pressure. Silica-gel column chromatography gave the desired compound in 97% yield (1.6 g); mp 123 °C (decomp); ^{1}H NMR ($CDCl_3$) δ=1.20 (m, 18H, $CH(CH_3)_2$), 2.60 (m, 5H, 2′H, and $CH(CH_3)_2$), 3.43 (m, 2H, 5′H), 3.82 (s, 6H, CH_3O), 4.18 (m, 1H, 4′H), 4.61 (m, 1H, 3′H), 6.32 (t, J=6 Hz, 1H, 1′H), 6.86 (m, 5H, 2,6-ArH of DMTr and one of OCH-CHO), 7.23—7.38 (m, 10H, ArH and one of OCH-CHO), 7.88 (s, 1H, 8H).

Found: C, 64.75; H, 6.54; N, 7.75%. Calcd for $C_{45}H_{51}N_5O_{11}$: C, 64.50; H, 6.14; N, 8.36%.

Pentachlorophenyl Nucleoside-3′-*O*-succinate (5). Typical procedure: 5′-*O*-DMTr-N^4-anisoyldeoxycytidine (680 mg, 1 mmol) was coevaporated with dry pyridine several times and dissolved in dry dichloromethane (10 ml). To the solution were added succinic anhydride (120 mg, 1.2 mmol), DMAP (12 mg, 0.1 mmol), and triethylamine (168 µl, 1.2 mmol). The reaction was performed at room temperature for 4 h. Then the mixture was transferred into a separatory funnel with dichloromethane (50 ml), and washed with an aqueous solution of citric acid (0.9%, 50 ml×2). The aqueous layers were back-extracted with CH_2Cl_2 (50 ml) and the organic layer was washed with water. These organic layers were combined and dried over Na_2SO_4. The solvent was removed under reduced pressure and pentachlorophenol (300 mg, 1.1 mmol) was added to the resulting foam. The mixture was rendered anhydrous by repeated coevaporation with dry pyridine and dissolved with dry CH_2Cl_2 (10 ml). DMAP (12 mg, 0.1 mmol) and DCC (310 mg, 1.5 mmol) were added to the solution and the mixture was stirred at room temperature for 1 h. Then the solid material was removed by filtration and washed with benzene. The filtrate and the washings were mixed and the solvent was removed under reduced pressure. Benzene (5 ml) was added and the resulting solid was filtered and washed with benzene (3 ml×3). The same procedure for removal of *N*,*N*′-dicyclohexylurea (evaporation, addition of benzene and filtration) was repeated one more time. The final filtrate and washings were evaporated and dissolved in CH_2Cl_2 (2 ml) and dropped into hexane–ether (7 : 1, v/v, 300 ml) with continuous stirring. The white precipitate was collected by filtration and dried in a desiccator (946 mg, 92%). This compound was characterized by comparison of its NMR spectrum with that of an authentic material.

Immobilization of Deoxyribonucleosides on Resins. General Procedure: A 3-aminopropyl resin (1 g) was rendered anhydrous by coevaporation with dry pyridine. An activated nucleoside (**5**) (0.1 mmol) and DMAP (0.01 mmol) dissolved in dry CH_2Cl_2 (10 ml) were added to the resin. Triethylamine (0.1 mmol) was added dropwise to the mix-

ture and the resulting suspension was slowly rotated for 1 h by the use of an evaporator. The resin was filtered and washed with CH_2Cl_2 and the unreacted amino group was blocked by the acetyl group by the reaction with acetic anhydride and DMAP in dry pyridine for 1 h. Finally, the nucleoside resin was obtained by filtration, washing with pyridine and CH_2Cl_2, and drying in vacuo. The loading amounts of nucleosides on the resin are listed in the text.

Synthesis of Oligomers on Resins. In the mannual preparation, the synthetic reactions were most conveniently carried out in a glass column with a sintered glass filter and a two way value which end was connected with a collection tube. During the drying step, the top of the column was joined to a vacuum pump and rotated by a rotary evaporator.

A reaction cycle consisted of the following steps: 1) A nucleoside resin in the column was treated with 1% TFA for 30 seconds repeatedly until the color of DMTr cation had disappeared, and the resin was washed with $CHCl_3$ (3 ml) three times. 2) The resin was rendered anhydrous by coevaporation with dry pyridine and mixed with a phosphodiester previously dried by coevaporation with dry pyridine. A condensing reagent was added to the mixture and the final concentration of the phosphodiester was about 0.1 M. The reaction was performed at room temperature with gentle rotation in the case of the CPG or with standing in the case of the polystyrene. 3) After the condensing reaction, all soluble reagents were washed out with pyridine several times, and the remaining 5′-hydroxyl group was blocked by the usual capping reaction, 4) The resin was washed with pyridine and then with $CHCl_3$ several times. Return to step 1, for the next cycle.

In the case of using the automated synthesizer, the resin was packed in a mini column and the reactions were carried out by continuous flow by a solvent pump. All reagents and solvents were delivered from reagent bottles being confirmed with the cycle program listed in the Table 7.

Yields of the condensing reactions were determined by estimation of released DMTr cation with a value of ε_{498}= 71700 (60% $HClO_4$: EtOH=3 : 2, v/v).

Deprotection of Oligomers by NaOH Method. The synthetic oligomer immobilized to a resin was treated with 0.1 M NaOH in pyridine-water (1 : 1, v/v) at room temperature for 1 h. The heterogeneous reaction mixture was applied to a mini column of Dowex 50 W×2 (pyridinium form, 1.5 ml of wet volume) and eluted with pyridine-water (1 : 1, v/v, 10 ml). All the eluent was collected and concentrated in vacuo and treated with concd aqueous ammonia at room temperature for 30 h and then at 50 °C for 1 h. The solution was carefully evaporated under reduced pressure, and in the case of the purification procedure by reversed phase HPLC, the resulting mixture was chromatographed on a μBondapak C_{18} (Waters) column and the desired fraction was collected and lyophilized to the solid. Then 5′ DMTr group was removed by treatment with 80% acetic acid for 15 min and concentrated by evaporation in vacuo. The oligomer was finally purified by rechromatography on a reversed phase HPLC column or by gel filtration chromatography with Shephadex G50 (2×150 cm, 0.05 M HCO_3^-). Purities of the oligomers obtained were analyzed by the HPLC or polyacrylamide gel electrophoresis (UV illumination) and the sequences were determined by the modified Maxam-Gilbert method.[15]

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