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INTRODUCTION OF THE 4,4'4"-TRIS(BENZOYLOXY)TRITYL GROUP INTO THE EXO AMINO GROUPS OF DEOXYRIBONUCLEOSIDES AND ITS PROPERTIES

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Abstract—The 4,4',4"-tris(benzoyloxy)trityl (TBTr) group was introduced into the amino groups of the common deoxyribonucleosides by means of the transient protection using the trimethylsilyl group. The TBTr group introduced onto the N^6-amino group of deoxyadenosine was found to have considerable retarding effects on the depurination when treated with acids.

In oligonucleotide synthesis, the amino groups of deoxyribonucleotides have been protected generally with acyl groups.[1] However, this protection mode becomes unfavorable in a large-scale synthesis of relatively long oligonucleotides required for physicochemical studies of DNA, since the lipophilicity of oligomers decreased with an increase in chain length so that separation of the coupling product on a silica gel column becomes difficult.[2] Therefore, more lipophilic groups have been introduced into deoxyribonucleotide residues.[3] Reverse phase column chromatography has also been applied to the synthesis of long oligomers.[4] In the case of the acyl-type of protection mode, different kinds of acyl groups have been employed for deoxycytidine (1), deoxyadenosine (2), and deoxyguanosine (3) because their removal conditions varied in each case.

Several research groups have pointed out the inevitable side reaction of the glycosyl bond cleavage in the case of deoxyadenosine derivatives upon acid treatment which was required for selective removal of the 4,4'-dimethoxytrityl (DMTr) group.[5] In order to solve this problem, milder acidic conditions have been searched for.[6] Recently, some improved protecting groups capable of suppressing the glycosyl bond rupture have also been reported.[7-10]

In this paper, we report that the highly lipophilic 4,4',4"-tris(benzoyloxy)-trityl (TBTr) group can be used as the common amino blockers for 1-3 and that this group stabilized considerably the glycosyl linkage.

In the previous paper,[11] we showed that the TBTr group can be introduced onto 5'-hydroxyls of various N-protected nucleosides and removed by the action of 1 M NaOH in aqueous dioxane-pyridine. This group was considerably resistant to acidic media. In fact, removal of the TBTr group from 5'-O-[4,4',4"-tris(benzoyloxy)-trityl]thymidine required 5 days on treatment with 80% acetic acid at room temperature. Compared with this fact, removal of the DMTr group from 5'-O-(4,4'-dimethoxytrityl)thymidine took only 10 min. Moreover, N-trityl groups bound to the exo amino functions are known to be more stable than those bound to the 5'-hydroxyls.[5a,12]

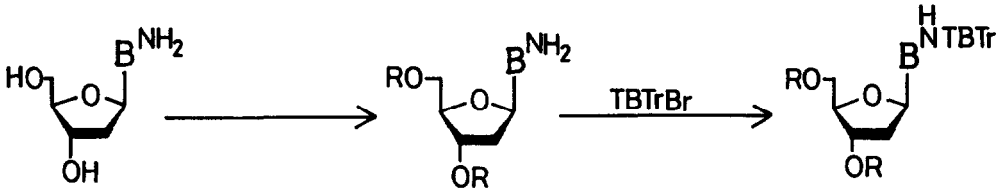
On the other hand, Zoltexicz[13] and other groups[14] suggested, on the basis of kinetic studies, that the depurination of deoxyadenosine occurs via a dication species. Matteucci[10] reported that unprotected deoxyadenosine was much more resistant to acid treatment than N-benzoyldeoxyadenosine. These facts indicate different mechanisms in depurination between N-acyl and N-unprotected deoxy-adenosines. It is likely that N-acylation decreases considerably the electron

density in the N^1-nitrogen so that the protonation occurs only on the 7-nitrogen atom. The great stability of unprotected deoxyadenosine to acidic media may be rationalized by the first protonaiton on the N^1-nitrogen, which leads to the electron-withdrawing effect on the whole purine ring as in the case of the phthaloyl group.[7] The inductive effect resulting from the cation charge was expected to be much stronger than that of the phthaloyl group. Therefore, we considered that, if a protecting group was designed in the manner where the N^1-nitrogen can be protonated, a stabilizing effect of the glycosyl bond as in the case of deoxyadenosine could be expected.

These facts and the above consideration led us to study the protection of the exo amino groups of 1-3 using the TBTr group.

First, we prepared 3',5'-protected deoxyribonucleosides (4-7) in order to search for suitable conditions for introduction of the TBTr group onto the amino groups of 1-3. As the hydroxyl protecting group, the t-butyldimethylsilyl or tetrahydropyranyl group was chosen. The conditions and results of the N-tritylation of 4-7 are summarized in Table I. In the case of 4, the use of the conventional conditions for the 5'-selective tritylation[11] using pyridine as the solvent and triethylamine as the scavenger of hydrogen bromide gave a 73% yield of the tritylated product (8). On the other hand, when the reaction was carried out in dimethylformamide (DMF) in the presence of silver nitrate[15] and 2,6-lutidine, 8 was obtained in an excellent yield of 91%. This silver ion catalyzed tritylation was also effected with deoxyguanosine derivative 6 and deoxyadenosine derivative 7, proving to be generally applicable. Although these reactions proceeded very rapidly, they required at least two equiv of TBTrBr. TLC suggested that TBTrBr was consumed competitively by an unknown side reaction giving rise to an inert nonnucleotidic material, which appeared higher than TBTrOH. Nevertheless, the reactions were completed substantially within 20 min.

In the previous paper,[11] we reported that tritylation of N^6-isobutyryldeoxyguanosine with TBTrBr in Et_3N-pyridine-CH_2Cl_2 gave an approximately 1:1 mixture of the desired 5'-O-TBTr derivative and a depurinated byproduct. It is noted that the silver catalyzed reaction provided exclusively the N-TBTr derivative without damage of the glycosyl bond.



1: B=Cy
2: B=Ad
3: B=Gu

4: B=Cy, R=TBDMS
5: B=Ad, R=TBDMS
6: B=Gu, R=TBDMS
7: B=Ad, R=THP

8: B=Cy, R=TBDMS
9: B=Ad, R=TBDMS
10: B=Gu, R=TBDMS
11: B=Ad, R=THP

TBDMS = t-butyldimethylsilyl
THP = tetrahydropyran-2-yl

Table I. Conditions and results of reaction of 4-7 with TBTrBr[a,b]

compd	solvent	temp	TBTrBr equiv	$AgNO_3$ equiv	base	equiv of base	product	yield %	mp (solv) °C
4	Py-CH_2Cl_2	r.t.	2		Et_3N	2.2	8	69	153-4 °C (hexane-ether)
	Py	65 °C	1.3		Et_3N	1.4		53	
	Py	65 °C	2		Et_3N	2.2		73	
	DMF	r.t.	1.3	1.3	luti.	1.3		72	
	DMF	r.t.	2	2	luti.	2		90	
5	DMF	r.t.	2	2	luti.	2	9	c	
6	DMF	r.t.	2	2	luti.	2	10	78	205-7 °C (CH_3CN)
7	DMF	r.t.	2	2	luti.	2	11	75	

[a] Each reaction was carried out for 1 h. [b] Py, DMF, and luti. refer to pyridine, dimethylformamide, and 2,6-lutidine, respectively. [c] The product 9 could not be separated from TBTrOH by silica gel column chromatography although tlc suggested ca. 80% formation of 9.

Next, in order to see if the TBTr group can be removed under the usual basic conditions used for oligonucleotide synthesis, several experiments were conducted with the N-TBTr compounds 8-11. Treatment of 8 with 2 M NaOH-dioxane-EtOH (2:2:1, v/v/v) for 10 min gave rapidly 4, which was isolated in 77% yield. In this reaction, partial elimination of the t-butyldimethylsilyl group was observed and the essential product was 4. Under the same conditions, the TBTr groups were removed completely from 10 and 11. It was also found that the conditions using tetramethylguanidium 2-pyridine-syn-carboxaldoximate[16] developed especially for removal of internucleotidic phosphate protecting groups were also effective for removal of the TBTr group. This oximate procedure resulted in complete deprotection of the TBTr group from 8-11 within 3 h. These results indicate that the TBTr group can be simultaneously removed along with the internal phosphate protecting groups in the phosphotriester approach and that post-treatment with concentrated ammonia is not neccessary as in the case of the use of N-acyl groups. Elimination of the latter treatment shortens the total time required for full deprotection. However, several attempts to remove selectively the t-butyldimethylsilyl or tetrahydropyranyl group from 9 or 11 were unsuccessful. The fluoride ion mediated deprotection procedure[17] caused simlultaneous elimination of the TBTr group to a considerable extent. Complete removal of the 3'- and 5'-O-tetrahydropyranyl groups from 11 was also difficult under acidic conditions such as 80% acetic acid and 0.5 M zinc bromide in CH_2Cl_2-iPrOH (85:15, v/v).[6d] It seems that the 3'-O-tetrahydropyranyl group still remained even on prolonged acid treatment whereupon the glycosidic bond cleavage began to take place.

For routine work in oligodeoxyribonucleotide synthesis, N-protected deoxyribonucleosides should be prepared by a simple procedure. Jones[18] and other workers[7,9] have recently reported a method for the synthesis of N-protected deoxyribonucleosides using the transient protection. Therefore, we have applied this method to the synthesis of 3',5'-free N-tritylated compounds (12-14). When 1 was treated with 2.4 equiv of trimethylsilyl chloride and 4.8 equiv of 2,6-lutidine in DMF followed by TBTrBr in the presence of silver nitrate, the desired product 12 was obtained in 91% yield. In the case of 2 and 3, the tritylations proceeded best at 0 °C to give 13 and 14 in 77 and 84% overall yields, respectively. The tritylations at ambient temperature resulted in lower yields of 13 and 14. The use of triphenylsilyl chloride as a silylating agent gave less formation of 12.

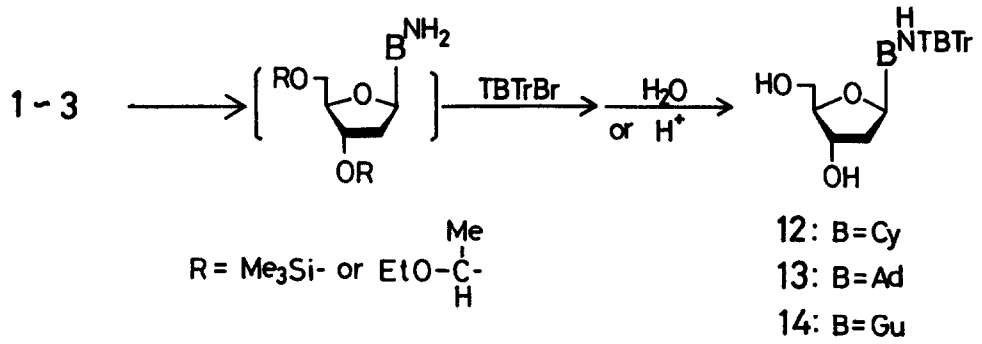


Table II. Conditions and results of reaction of 1-3 with TBTrBr.

compd	silylation[a] Me_3SiCl equiv	lutidine equiv	TBTrBr equiv	$AgNO_3$ equiv	tritylation[b] lutidine equiv	temp	product	yield %	mp (solv)
1	2.4	5.8	2	5.4	5.4	r.t.	12	91	172-4 °C (EtOH-H_2O)
2	2.4	4.8	2	4.4	4.4	r.t.	13	72	198-202 °C (CH_3CN)
	2.4	4.8	2	4.4	4.4	0 °C		77	
3	2.4	4.8	2	4.4	4.4	r.t.	14	67	213-214 °C (MeOH)
	3.4	6.8	2	5.4	5.4	0 °C		84	

[a]The silylation was carried out at room temperature for 1 h.
[b]The tritylation was carried out for 1h.

On the other hand, the 1-ethoxyethyl group[19] was also useful as a transient blocking group. In this case, 2% p-toluenesulfonic acid in CH_2Cl_2-MeOH (7:3, v/v) was employed for removal of the 1-ethoxyethyl group after the tritylation was completed. Thus, this procedure gave 12 in 75% yield from 1 but was not applied to the synthesis of 13 and 14 since the 1-ethoxyethylation of the exo amino groups simultaneously occured to a significant extent.[9]

Finally, we examined the stability of the glycosidic bond of 13 under acidic conditions. As discussed above, it was, indeed, confirmed that the glycosyl C-N bond of 13 was very stable towards acids such as 2% dichloroacetic acid (DCA) in methylene chloride and 80% acetic acid. Compared with the benzoyl group, the TBTr group had the remakable retarding effect on the depurination. Table IV implies that the glycosyl bond of 13 was 4 times more stable in 2% DCA/CH_2Cl_2 than that of N^6-benzoyldeoxyadenosine (dBzA). In this medium, the C-N bond of N^6-phthaloyl-deoxyadenosine (dPhtA) was 40 times more stable than that of dBzA. Therefore, the order of the N-protecting groups in the retarding effect on the depurination is Pht > TBTr > Bz in 2% DCA/CH_2Cl_2. However, we found that the above order changed in 80% acetic acid. In the latter acid solution, the TBTr group stabilize the C-N bond of deoxyadenosine 5 times more effectively than the phthaloyl group. The reason for the difference in stabilizing effect of the N-protecting groups between the two acid solutions is not clear. Further study must be needed for the explanation. From a steric point of view, it seems that the presence of the neighboring bulky TBTr group makes the N^1- and N^7-nitrogens less accessible to the protonation due to steric hindrance as illustrated in Figure 1.

Table III. ^{1}HNMR Spectra and Elemental Analyses of Comounds 4-14

Compd	^{1}H NMR in $CDCl_3$ δ, ppm	elemental analysis formula calcd C, H, N (%) Found C, H, N (%)	Rf value on TLC CH_2Cl_2-MeOH 9:1 20:1
4	0.08 (6, s, CH_3Sia), 0.12 (6, s, CH_3Sib), 0.91 (9, s, CH_3Ca), 0.95 (9, s, CH_3Cb), 2.08 (1, m, 2'-Ha), 2.40 (1, m, 2'-Hb), 3.88 (3, m, 4'-H and 5'-H), 4.39 (1, q, J = 5 Hz, 3'-H), 5.79 (1, d, J = 8 Hz, 5'-H), 6.27 (1, t, J = 6 Hz, 1'-H), 7.05 (2, br, NH_2)	$C_{21}H_{41}N_3O_4Si_2 \cdot 1/4H_2O$ 54.80, 9.09, 9.13 54.61, 9.16, 9.39	0.38, 0.07
5	0.09 (12, s, CH_3Si), 0.91 (18, s, CH_3C), 2.54 (2, m, 2'-H), 3.83(2, t, J = 3.5 Hz, 5'-H), 4.00 (1, t, J = 3.5 Hz, 4'-H), 4.60 (1, m, 3'-H), 6.36 (2, br, NH_2), 6.45 (1, t, J = 6 Hz, 1'-H), 8.14 (1, s, 2-H), 8.35 (1, s, 8-H)	$C_{22}H_{41}N_5O_3Si_2$ 55.08, 8.61, 14.60 54.90, 8.79, 14.90	0.56, 0.16
6	0.17 (s, s, CH_3Sia), 0.22 (6, s, CH_3Sib), 0.97 (18, s, $(CH_3)_3C$), 2.62 (2, m, 2'-H), 3.95 (2, m, 5'-H), 4.15 (1, m, 4'-H), 4.74 (1, m, 3'-H), 6.55 (1, m, 1'-H)	$C_{22}H_{41}N_5O_4Si_2 \cdot 1/4H_2O$ 52.82, 8.36, 14.00 53,19, 8.48, 14.33	0.33, 0.03
7	1.40-2.00 (12, m, CH_2 of THP), 2.62 (2, m, 2'-H), 3.30-4.40 (7, m, 4'-H, 5'-H, and CH_2O of THP), 4.60 (3, m, 3'-H and O-CH-O), 6.15 (1, s, N-H), 6.48 (1, 1'-H, m), 8.20 (1, s, 2-H), 8.34 (1, s, 8-H)	$C_{20}H_{29}N_5O_5 \cdot 1/4H_2O$ 56.72, 6.94, 16.34 57.05, 7.31, 15.99	0.80, 0.60
8	0.02 (6, s, CH_3Sia), 0.08 (6, s, CH_3Sib), 0.82 (9, s, CH_3Ca), 0.90 (9, s, CH_3Cb), 2.10 (1, m, 2'-H), 2.42 (1, m, 2'-Hb), 3.62-4.00 (3, m, 4'-H and 5'-H), 4.35 (1, m, 3'-H), 5.10 (1, d, 5-H), 6.28 (1, t, J = 6 Hz, 1'-H), 6.84 (1, br, NH), 7.17-7.68 (23, m, ArH), 7.82 (1, d, J = 8 Hz, 6-H), 8.16-8.30 (6, m, ArH)	$C_{61}H_{67}N_3O_{10}Si_2$ 69.23, 6.38, 3.97 69.25, 6.40, 3.94	0.87, 0.54
10	0.02 (12, s, CH_3Si), 0.84 (18, s, CH_3C), 2.00 (2, m, 2'-H), 3.70 (2, m, 5'-H), 3.88 (1, m, 4'-H), 5.70 (1, t, J = 5 Hz, 1'-H), 7.08- 7.68 (22, m, ArH), 7.71 (1, s, 8-H), 8.15-8.25 (6, m, ArH)	$C_{62}H_{67}N_5O_{10}Si_2$ 67.80, 6.15, 6.38 68.17, 6.25, 6.17	0.69, 0.12
11	1.40-2.00 (12, m, CH_2 of THP), 2.66 (2, m, 2'-H), 3.40-4.44 (7,m, 4',5'-H and CH_2O of THP), 4.40-4.82 (3, m, 3'-H and O-CH-O), 6.50 (1, m, 1'-H), 6.89 (1, s, NH), 7.10-7.88 (21, m, ArH), 8.12-8.45 (8, m, ArH)	$C_{60}H_{55}N_5O_{11}$ 70.51, 5.42, 6.85 70.47, 5.56, 6.79	0.91, 0.74
12	2.37 (2, m, 2'-H), 3.78 (2, m, 5'-H), 3.98 (1, m, 4'-H), 4.50 (1, m, 3'-H), 5.15 (1, d, J = 7.6 Hz, 6-H), 6.10 (1, t, J = 6 Hz, 1'-H), 7.10-7.75 (22, m, 5-H and ArH), 8.13-8.23 (6, m, ArH), 8.58 (1, br, NH)	$C_{49}H_{39}N_3O_{10} \cdot H_2O$ 69.41, 4.87, 4.96 69.41, 4.54, 4.94	0.41, 0.05
13	2.35 (1, m, dd, J_{gem} = 13 Hz, J = 6 Hz, 2'-Ha), 3.00 (1, m, 2'-Hb), 3.66 (1, d, J_{gem} = 12 Hz, 5'-Ha), 3.92 (1, d, J_{gem} = 12 Hz, 5'-Hb), 4.18 (1, m, 4'-H), 4.74 (1, m, 3'-H), 6.35 (1, dd, J = 5 Hz, J = 9 Hz, 1'-H), 7.10-7.75 (21, m, ArH), 7.93 (1, s, 2-H), 8.08 (1, s, 8-H), 8.15-8.30 (6, m, ArH)	$C_{50}H_{39}N_5O_9 \cdot H_2O$ 68.89, 4.74, 8.03 68.97, 4.35, 8.19	0.57, 0.12
14	2.10 (2, m, 2'-H), 3.40 (2, br, OH), 3.64 (2, m, 5'-H), 3.88 (1,m, 4'-H), 4.42 (1, m, 3'-H), 5.70 (1, m, 1'-H), 6.86-7.10 (6, m, ArH), 7.30-7.80 (16, m, ArH and 8-H), 8.04-8.20 (6, m, ArH)	$C_{50}H_{39}N_5O_{10}$ 69.39, 4.52, 8.05 69.35, 4.57, 7.99	0.32, 0.02

[a]The solvent used was d^5-pyridine.

Table IV. Depurination of N-protected deoxyadenosine derivatives under acidic conditions.

Compd	2% DCA/CH_2Cl_2 $t_{1/2}$ (comp.)	1% TFA/CH_2Cl_2 $t_{1/2}$	80% AcOH $t_{1/2}$
dA	stable	stable	stable
dA^{bz}	5 min (20 min)	5 min (15 min)	40 min
dA^{Pth}	2.5 h (18 h)	35 min (5 h)	2 h
dA^{TBTr} 13	20 min (1 h)	6 min (25 min)	5 h

Table V. Stability of the N-TBTr group on compounds 12 and 14 under acidic conditions.

compd	2% DCA/CH_2Cl_2 $t_{1/2}$	1% TFA $t_{1/2}$	80% AcOH $t_{1/2}$
dC^{TBTr} 12	$\gg$ 48 h	stable (for 24 h)	stable (for 24 h)
dG^{TBTr} 14	20 h	$>$ 48 h	insoluble

Next, the stability of the TBTr groups of 12 and 14 was examined in the acid solutions. The TBTr group of 12 was remarkably stable under acidic conditions as suggested from Table V. The TBTr group of 14 was eliminated to an extent of 50% when treated with 2% dichloroacetic acid in CH_2Cl_2 for 20 h. After this time, the depurination of 14 was observed to an unnegligible extent. These results indicate that the N-TBTr derivatives 12-14 can be used as key intermediates for the oligodeoxyribonucleotide synthesis where a rapid deprotection procedure is devised.

As far as the steric effect and lipophilicity are concerned, the TBTr group is superior to the cyclic and acyclic amidine type of protecting groups recently reported by Caruthers[8] and Matteucci.[10] In addition, the TBTr group was stable in aqueous pridine in which the phthaloyl group was hydrolyzed gradually with its ring opening. Our preliminary study on oligodeoxyribonucleotide synthesis using the TBTr group showed that the DMTr group was selectively removed in the presence of the N-TBTr groups. These results will be reported in the near future.

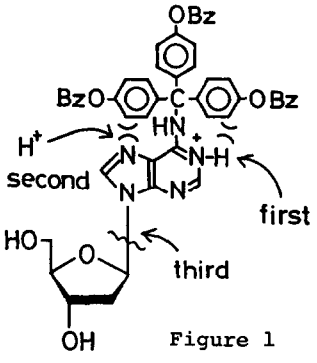


Figure 1

Experimental

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^1H NMR spectra were recorded at 100 MHz on a JNM-PS-100 spectrometer. UV spectra were obtained on Hitachi 124 spectrophotometer. Reagent grade pyridine was distilled after being refluxed over p-toluenesulfonyl chloride for several hours, redistilled over calcium hydride after being refluxed for several hours, and stored over 3A molecular sieves. DMF was distilled and stored over 3A molecular sieves. Column chromatography was performed by using silica gel C-200 purchased from Wako Co. Ltd., and minipump for a goldfish basin was conveniently used to gain a medium pressure for rapid chromatographic separation. Thin layer chromatography was performed on precoated TLC plates silica gel 60 F-254 (Merck). The Rf values of the protected nucleoside derivatives were measured after development with the following solvent systems: Solvent A (CH_2Cl_2-MeOH, 9:1, v/v); Solvent B (CH_2Cl_2-MeOH, 20:1, v/v), (9:1, v/v). TBTrBr was prepared from TBTrOH by the previously reported method.[11] TBTrOH was prepared by a modification of Gomberg's method.[20] The details of its preparation were described below. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta. Analytically pure samples of nucleoside derivatives were obtained by reprecipitation from methylene chloride with hexane. No attempts to crystallyze the powdery materials from appropriate solvents have been made.

4,4',4"-Tris(benzoyloxy)tritylcarbinol. Rosolic acid (100 g, 0.344 mol) was dissolved in pyridine (300 mL) and benzoyl chloride (250 mL, 2.15 mol) was added with cooling. The resulting mixture was refluxed for 1 h and then ice-water (200 g) was added. A 5% $NaHCO_3$ solution was added until the evolution of CO_2 gas ceased. The aqueous solution was extracted with CH_2Cl_2 (3 x 200 mL). The organic layers were combined, dried over Na_2SO_4, and filtered. The filtrate was evaporated and the residue was coevaporated several times with toluene to remove the last traces of pyridine. The resulting solid was recrystallized from benzene to give a slightly yellowish crystals of TBTrOH (146.1 g, 58%): mp 101-103 °C: ^1H NMR ($CDCl_3$): δ 3.13 (1, br, OH), 7.13 (6, d, J = 9 Hz, ArH), 7.10-7.70 (15, m, ArH), 8.10 (6, m, ArH). Anal. Calcd for $C_{40}H_{28}O_7 \cdot 1/4H_2O$: C, 76.85; H, 4.60. Found: C, 76.75; H, 4.63. Gomberg[20] reported that TBTrOH tended to form adducts with various solvents used for recrystallization. However, the crystals obtained in this experiment were found to be free from benzene but contain 1/4 H_2O. Therefore, this compound has a different mp from that (190-191 °C) reported by Gomberg.

3',5'-O-Bis(t-butyldimethylsilyl)deoxycytidine (4). Deoxycytidine hydrochloride (2.64 g, 10 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and finally suspended in dry pyridine (100 mL). To the suspension was added t-butyldimethylsilyl chloride (3.62 g, 24 mmol). The mixture was stirred at room temperature for 2 h. However, the reaction proceeded very slowly. Therefore, the silyl chloride (1.51 g, 10 mmol) was added and the mixture was stirred at room temperature for 2 h and then at 60 °C for 1 h. At this time, the reaction was not yet complete due to the insolubility of the starting nucleoside in this medium. Finally, the silyl chloride (1.51 g, 10 mmol) was added and the mixture was stirred vigorously at 35-40 °C for 15 h. Then, water (50 ml) and triethylamine (5 mL) were added and the aqueous solution was extracted several times with CH_2Cl_2. The organic extracts were combined and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the residue was coevaporated three times with toluene and chromatographed on a silica gel column to give 4 (3.87 g, 85%): mp 185-6 °C (CH_3CN).

3',5'-O-Bis(t-butyldimethylsilyl)deoxyadenosine (5). Deoxyadenosine monohydrate (2.51 g, 9.3 mmol) was rendered anhydrous by repeated coevaporations with dry DMF and dissolved in dry DMF (150 mL). To the solution were added imidazole (3.27 g, 48 mmol) and t-butyldimethylsilyl chloride (3.12 g, 24 mmol). After the mixture was stirred at room temperature for 6 h and then at 60 °C for 1 h. The mixture was partitioned between CH_2Cl_2 (300 mL) and 5% aqueous $NaHCO_3$ (300 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ (2 x 100 mL) and then water (2 x 100 mL). Each washing was back-extracted with the same CH_2Cl_2 (50 mL) in another separatory funnel. The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and concentrated to a gum under reduced pressure. The gum was chromatographed on a silica gel to give 5 (4.12 g, 92%): mp 131-132 °C (CH_3CN).

3',5'-O-Bis(t-butyldimethylsilyl)deoxyguanosine (6). Deoxyguanosine monohydrate (2.67 g, 9.4 mmol) was rendered anhydrous as described in the case of 4 and suspended in dry pyridine (100 mL). To the suspension was added t-butyldimethylsilyl chloride (1.51 g, 10 mmol), and the resulting mixture was stirred at room temperature for 1.5 h. Since the reaction was not complete, the mixture was treated two times with the silyl chloride (1.51 g, 10 mmol) in the same manner as described in the case of 4. Then, water (100 mL) was added and the white solid precipitated was collected by filtration. The filtrate was further extracted with CH_2Cl_2 (150 mL), dried over Na_2SO_4, and filtered. The CH_2Cl_2 was concentrated to half the volume whereupon the white precipitate appreared. This precipitate was collected by filtration, mixed with the first solid, and recrystallized from ethanol-water (9:1, v/v, 800 mL) to afford 6 (3.71 g, 80%) as crystals: mp > 250 °C (EtOH).

3',5'-O-Bis(tetrahydropyran-2-yl)deoxyadenosine (7). To a mixture of deoxyadenosine monohydrate (1.26 g, 4.7 mmol) and dihydropyran (9 mL, 100 mmol) in dry dioxane (50 mL) was added p-toluenesulfonic acid monohydrate (0.38 g, 2 mmol). The mixture was stirred at room temperature for 1.5 h and then p-toluenesulfonic acid monohydrate (1.83 g, 3.1 mmol) was added. After the resulting mixture was stirred for 30 min, concentrated ammonia (25%, 0.6 mL) was added. The aqueous solution was extracted with CH_2Cl_2 (3 x 50 mL). After the solvent was removed under reduced pressure, the residue was chromatographed on a silica gel column to afford 7 (1.43 g, 70%) as syrup.

General Procedure for the N-Tritylation of 4-7. Method A: Compound 4 (228 mg, 0.5 mmol) was rendered anhydrous by repeated coevaprtations with dry pyridine and finally dissolved in dry pyridine (4 mL) or pyridine-CH_2Cl_2 (1 mL-1 mL). To the solution were added successively triethylamine and TBTrBF as listed in Table I. The resulting mixture was vigorously stirred at room temperature or at 65 °C for the times tabulated in Table I. Then, water was added and the solution was extracted with CH_2Cl_2 (3 x 20 mL). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column with hexane-CH_2Cl_2 to afford 8.
Method B: Compound 4, 6, or 7 (0.5 mmol) was rendered anhydrous two times by

coevaporations with dry DMF and finally dissolved in dry DMF (5 mL). To the solution were added successively 2,6-lutidine, silver nitrate, and TBTrBr. The detailed conditions are summarized in Table I. The resulting mixture was vigorously stirred at room temperature for 1 h. The reaction was quenched by addition of water and the precipitate was removed by filtration. The filtrate was partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The CH_2Cl_2 extract was washed further with water (4 x 50 mL). Each washing was back-extracted with the same CH_2Cl_2 (20 mL) put in another separatory funnel. The organic extracts were combined and worked up as described in Method A to give the N-tritylated compounds as listed in Table I.

Conversion of 8 to 4 by Alkaline Treatment. To a solution of 8 (212 mg, 0.2 mmol) were added ethanol (5 mL) and 2 M NaOH (2.5 mL). The resulting mixture was stirred at room temperature for 10 min. At this time the TBTr group was completely removed as evidenced by appearance of 4, rosolic acid, and benzoic acid on TLC. The mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried over Na_2SO_4, evaporated, and chromatographed on silica gel to give 4 (70 mg, 77%).

Oximate Treatment of 8-10. A sample of 8, 9, or 10 (10 μmol) was dissolved in a 1 M solution of tetramethylguanidium 2-pyridine-syn-carboxaldoximate in dioxane-water (7:1, v/v, 600 uL). Aliquots from the mixture were taken at appropriate times and analyzed by thin layer chromatography on silica gel using Solvents A and B. The results are described in the text.

Attempts to Remove the t-Butyldimethylsilyl or Tetrahydropyranyl Group from 8 or 11. A: Compound 8 (390 mg, 0.367 mmol) was dissolved in a THF solution (33%, 0.625 mmol) of tetrabutylammonium fluoride. The reaction was monitored by thin layer chromatography on silica gel. After 60 min, TLC exhibited a very complicated mixture containing at least six spots other than 8.

B: Compound 11 (10 mg, 9.6 μmol) was treated with 80% acetic acid (1 mL). Both the treatments showed similar results but the former gave 13 faster than the latter. However, the starting material remained unchanged (10-20%) and only 30-50% conversions of 11 to 13 were achieved even after 20 h.

General Procedure for the Synthesis of 12-14. An appropriate deoxyribonucleoside (1 mL) was rendered anhydrous by coevaporations with dry DMF and finally suspended or dissolved in dry DMF (8 mL). To the resulting mixture were added 2,6-lutidine and trimethylsilyl chloride. The mixture was stirred vigorously for 1 h. At this time, the 3',5'-O-bistrimethylsilylation was complete. Then, additional 2,6-lutidine, silver nitrate, and TBTrBr were added successively to the mixture and the resulting solution was stirred vigorously for 1 h. The precipitate was removed by filtration and washed with DMF (20 mL). Water (5 mL) was added to the filtrate to remove the trimethylsilyl groups. After being stirred for 40 min (60 min in the case of 3), the solution was partitioned between CH_2Cl_2, (50 mL) and water (50 mL). The same workup as described in General Procedure for the Synthesis of 4, 6, or 7 (Method B) gave the N-tritylated products as listed in Table II.

Method for the Synthesis of 12 Using the 1-Ethoxyethyl Group as Transient Blocker. Deoxycytidine hydrochloride (264 mg, 1 mmol) was rendered anhydrous by ceovaporations with dry DMF and finally suspended in dry DMF (5 mL). To the suspension were added ethyl vinyl ether (1.91 mL, 20 mmol) and trifluoroacetic acid (15.4 μL, 0.2 umol). The mixture was stirred vigorously for 1.5 h and then evaporated under reduced pressure to remove the excess vinyl ether and acid. The residue was dissolved in dry DMF (3 mL), and 2,6-lutidine (221 μL, 3 mmol), silver nitrate (510 mg, 3 mmol), and TBTrBr (1.44 g, 2 mmol) were successively added. The resulting mixture was stirred for 1 h, and then filtered and washed with DMF (3 mL). The filtrate was treated with 2% toluenesulfonic acid monohydrate in CH_2Cl_2-MeOH (7:3, v/v, 10 mL) at room temperature for 2 h. The mixture was partitioned between CH_2Cl_2 (30 mL) and 5% $NaHCO_3$ (30 mL). After the usual workup, chromatography gave 12 (618 mg, 75%).

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