The *tert*-Butyldimethylsilyl Group as a Protecting Group in Deoxynucleosides

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tert-Butyldimethylsilyl chloride reacts selectively with hydroxyl groups in nucleosides and preferentially with the 5′-hydroxyl of deoxynucleosides. The *tert*-butyldimethylsilyl group can be removed under conditions which do not affect other commonly used acid or base labile protecting groups, yet it is stable to phosphorylation conditions. This allows for the synthesis of a variety of protected deoxynucleosides in good yields and introduces a versatile new protecting group to the nucleoside and nucleotide field.

Le chlorure de *tert*-butyldiméthylsilyle réagit sélectivement avec les groupes hydroxyles des nucléosides et préférentiellement avec les hydroxyl-5′ des désoxynucléosides. Tout en étant stable dans les conditions de phosphorylation, le groupe *tert*-butyldiméthylsilyle peut être enlevé dans des conditions qui n'affectent pas d'autres groupes protecteurs utilisés couramment et qui sont labiles en milieux acide ou basique. Ceci permet d'effectuer, avec de bons rendements, la synthèse d'un grande nombre de déoxynucléosides protégés et d'introduire un nouveau groupement protecteur versatile au domaine des nucléosides et des nucléotides. [Traduit par le journal]

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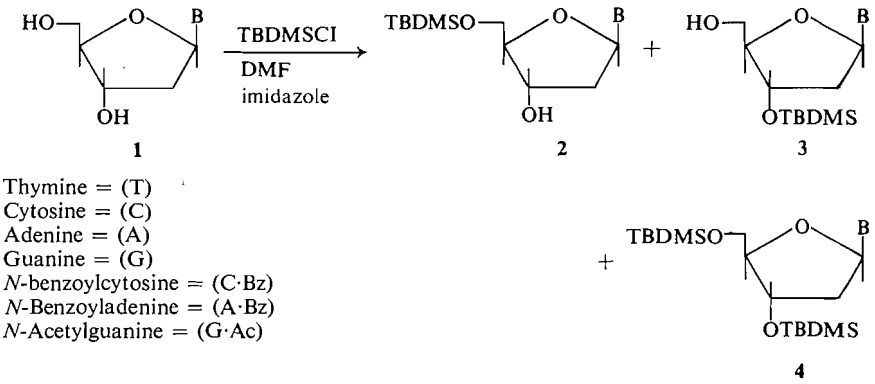
During our investigations into the synthesis and indentification of nucleosides and nucleotides (1, 2) we have been seeking versatile hydroxyl-protecting groups. We describe in this report the use of the *tert*-butyldimethylsilyl (TBDMS) group for protecting hydroxyl functions in deoxynucleosides (3) and the subsequent synthesis of a number of nucleoside derivatives which have not previously been easy to prepare.

tert-Butyldimethylsilyl chloride (TBDMSCL) is prepared by the reaction of *tert*-butyllithium with dichlorodimethylsilane (4). Corey and Venkateswarlu have described the use of TBDMSCL (in DMF at 35 °C in the presence of imidazole) for protecting the hydroxyl groups of a number of alcohols of interest in the synthesis of prostaglandins (5). We have applied these same conditions to the deoxynucleosides and find that: (*a*) TBDMSCL reacts preferentially with the 5′-hydroxyl group; (*b*) the reaction is rapid even at room temperature and the extent of the reaction and distribution of products depends on the amount of imidazole present; (*c*) even in the presence of excess TBDMSCL reaction occurs preferentially with hydroxyl groups and not with amino groups; (*d*) the TBDMS group is stable to phosphorylation conditions; and (*e*) the TBDMS group can be removed with $(n\text{-butyl})_4NF$ in tetrahydrofuran (THF) as described by Corey and Venkateswarlu (5) without affecting other acid or base labile protecting groups on the nucleoside. This has facilitated the preparation of a number of protected nucleosides.

The preparation of the silyl derivatives (Scheme 1) simply requires dissolving the nucleoside, TBDMSCL and imidazole in *N*,*N*-dimethylformamide (DMF). Our initial experiments were carried out at 37 °C. However, we find the reactions are over very quickly (within 30 min) at room temperature with no significant change in yields (Table 1). Further there appears to be little change in product composition for reactions times of 1–24 h.

The amount of imidazole present is important in terms of total yield and also ratio of products (Table 1). We find that for thymidine and *N*-protected nucleosides a ratio of nucleoside: TBDMSCL:imidazole near 1:1.1:2.2 gives the highest yields of 5′-isomers **2**. Reducing the amount of imidazole decreases the total yield of products and increases the ratio of **3**:**2**. Imidazole or other catalyst is essential to the reaction since the absence of imidazole in DMF gives ~5% total yield. However if pyridine is used as solvent in place of DMF, the pyridine also effectively replaces imidazole as catalyst (Table 1). For nucleosides possessing a free amino group the above ratio of reagents generally changes to 1:2.2:4:4 for optimum yields of **2**. However, even under conditions where we obtain di-*O*-silylated nucleosides in



SCHEME 1

TABLE 1. Reactions of deoxynucleosides with TBDMSCL

Starting material (1 mmol)	Ratio 1:TBDMSCL:imidazole	Solvent (1 ml)	Temperature (°C)	Time (h)	Products (% yields)* 1	2	3	4	Other† (mg)
1*a*	1:1.1:1.1	DMF	37	24	14	33	20	28	
1*a*	1:1.1:2.2	DMF	37	24	3	75	4	15	
1*a*	1:1.1:2.2	DMF	37	1	10	75	2	14	
1*a*	1:1.1:2.2	DMF	22	1	10	73	1	15	
1*a*	1:1.5:3.0	DMF	37	15	5	48	3	43	
1*a*	1:1.1:4.4	DMF	37	24	4	76	5	14	
1*a*	1:1.1:0	DMF	37	24	94	1.4	3.1	1	
1*a*	1:1.1:0	Pyridine	37	24	15	64	1.5	13	
1*b*·HCl	1:1.1:2.2	DMF	22	15	32	55	6	3	
1*b*·HCl	1:2.2:4.4	DMF	22	15	<1	88	3	10	
1*b*·HCl	1:4.4:8.8	DMF	22	15	—	—	—	93	8
1*c*	1:1.1:1.1	DMF	22	15	80	15	1.4	<1	
1*c*	1:2.2:4.4	DMF	22	15	<1	80	1.6	17	
1*c*	1:4.4:8.8	DMF	22	15	—	19	<1	71	57
1*d*	1:1.1:2.2	DMF	22	15	45	32	12	5	
1*d*	1:2.2:4.4	DMF	22	15	2	76	4	16	
1*d*	1:3.3:6.6	DMF	22	15	—	—	—	97	
1*e*	1:1.1:2.2	DMF	37	20	22	59	1	16	
1*e*	1:1.1:2.2	DMF	22	15	10	62	8	18	
1*e*	1:2.2:4.4	DMF	22	15	—	2	2	96	
1*f*	1:1.5:3.0	DMF	22	2	14	68	6	8	
1*g*	1:1.1:2.2	DMF	22	15	23	58	5	12	

*Actual experimental yields. Products isolated by t.l.c.
†In both cases where polysilyated products were isolated the mass spectrum indicated three silyl groups on the nucleoside.

90% yields we seldom isolated trisilylated nucleosides (*N*-substitution). This preference of TBDMSCL for reaction with the hydroxyl groups of nucleosides adds considerable versatility to nucleoside protecting groups and allows easy manipulation of a variety of protecting groups.

We have generally found that nucleosides substituted on the 3′-hydroxyl group move more rapidly on t.l.c. than the 5′-isomers. In our reactions to produce the 5′-TBDMS derivatives we expected, because of the bulk of the silylating agent, that the 5′-isomer would predominate. In six out of the seven cases where we observed two monosilylated products from the reaction of TBDMSCL with a nucleoside (Scheme 1) the slower moving (5′) isomer predominated over the faster moving (3′) isomer (see Tables 1, 2). The exception was deoxycytidine where **3***b* moved slightly slower on t.l.c. than **2***b* in the 8:2 solvent (see Experimental).

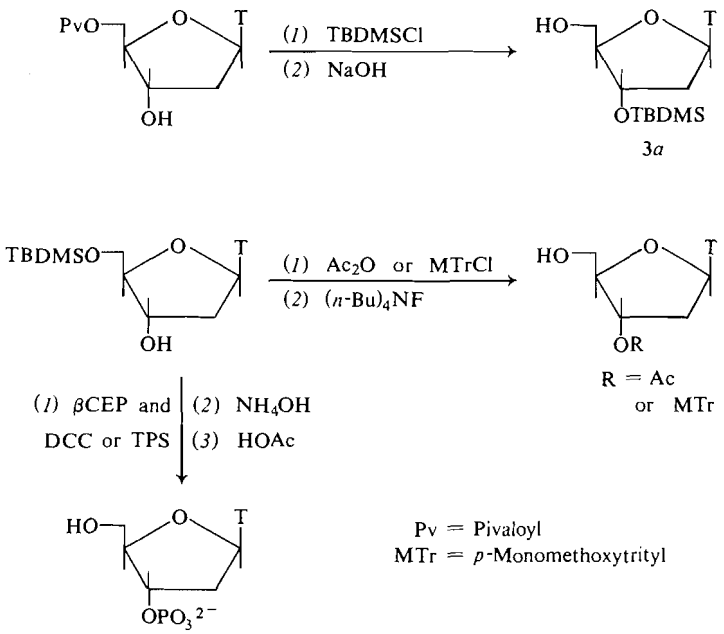
TABLE 2. Physical properties of silylated nucleosides

Compound	M.p. (°C)	Crystallized from	λ_{max} (EtOH) (nm)	Analysis						R_f values†		
				Calculated			Found					
				C	H	N	C	H	N	Ethyl acetate	THF	8:2‡
2a	198–199	ethanol–water	267	53.91	7.92	7.86	54.05	8.03	7.81	0.50	0.80	0.74
3a	83–84	ethanol–water	267	53.91	7.92	7.86	53.81	8.16	7.72	0.58	0.84	0.79
4a	144–145	hexane	267	53.13	8.99	5.95	56.06	9.09	5.86	0.73	0.90	0.84
2b	240–242	ethanol–water	274,240sh	52.76	7.96	12.31	52.64	8.04	12.18	0.00	0.22	0.46
3b	163–165	ethanol–water	273,238sh	52.76	7.96	12.31	52.60	8.10	12.22	0.00	0.22	0.40
4b	188–189	ether	274,240sh	55.34	9.07	9.22	55.26	9.09	9.16	0.12	0.35	0.64
2c	165–166	ether–hexane	260	52.58	7.51	19.01	52.58	7.51	19.01	0.23	0.71	0.70
3c	216–217	ether–hexane	260	52.58	7.51	19.01	52.45	7.50	19.22	0.36	0.77	0.76
4c	132.5–133	hexane	260	55.07	8.61	14.60	54.97	8.64	14.51	0.55	0.80	0.77
2d	dec. > 230	ethanol–water	255,275sh	50.37	7.13	18.36	50.09	7.05	18.08	0.00	0.08	0.30
3d	82–84	ethanol–water	255,273sh	50.37	7.13	18.36	50.45	7.08	18.12	0.00	0.08	0.50
4d	dec. > 265	ethanol	255,275sh	53.30	8.34	14.13	53.25	8.41	14.01	0.07	0.25	0.56
2e	130–132	THF–hexane	305,260	59.30	7.01	9.43	59.50	7.20	9.26	0.22	0.70	0.70
3e	*	*	305,260	59.30	7.01	9.43	59.43	6.93	9.33	0.45	0.80	0.80
4e	138–139	*	305,260,236sh	60.07	8.10	7.51	60.22	8.18	7.65	0.55	0.91	0.84
2f	98–99	water	280,260sh,253sh	58.82	6.65	14.91	59.12	6.78	14.76	0.26	0.79	0.86
3f	128–131	ethanol	280,260sh,253sh	58.82	6.65	14.91	59.08	6.85	15.08	0.46	0.83	0.90
4f	67–68	*	280,260sh,253sh	59.65	7.77	11.99	59.60	7.71	12.12	0.80	0.82	0.90
2g	123–125	ethanol–water	280,260,255	51.05	6.90	16.54	50.75	6.82	16.65	0.05	streak	0.50
3g	191–192	ethanol	280,260,255	51.05	6.90	16.54	50.82	6.73	16.55	0.05	streak	0.58
4g	105–107	ethanol	280,260,255	53.60	8.06	13.02	53.47	8.02	13.12	0.16	streak	0.83

*Samples obtained as dry solids and/or in small quantities and usually did not show sharp melting points. Mass spectrum, t.l.c., and elemental analysis indicated compounds were pure.

†Eastman chromatogram sheets 6060, silica gel with indicator.

‡8:2 refers to a solvent composed of chloroform:ethanol (8:2, v/v).



SCHEME 2

To confirm the identity of the isomers **2***b* and **3***b*, we prepared **3***b* by an alternate route. $O^{5'}$-Acetyl-*N*-benzoyldeoxycytidine was treated with TBDMSCL followed by removal of the acetyl group with NH_4OH and the benzoyl group with hydrazine hydrate. This produced **3***b* identical to the slower moving product from the reaction of **1***b* with TBDMSCL.

To confirm the identity of the isomers **2***a* and **3***a* of thymidine we treated $O^{5'}$-pivaloylthymidine with TBDMSCL and hydrolyzed the intermediate with 0.5 *N* NaOH. The product was isolated by t.l.c. and found to be identical to the faster moving monosilylated thymidine which is **3***a*. We have detected no isomerization of **2***a* and **3***a* under these conditions. Furthermore **2***a* was reacted in separate experiments with acetic anhydride and monomethoxytrityl chloride (Scheme 2). Removal of the silyl group with $(n\text{-Bu})_4NF$ in THF produced the known compounds $O^{3'}$-acetylthymidine (6) and $O^{3'}$-monomethoxytritylthymidine (**1***b*) in 95 and 94% isolated yields respectively.

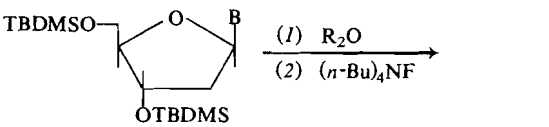
Further proof of the identities of **2***a* and **3***a* was obtained by phosphorylation and enzymatic analysis of the products. Compound **2***a* was phosphorylated using β-cyanoethylphosphate (βCEP) and either *N*,*N*′-dicyclohexylcarbodiimide (DCC) or triisopropylbenzenesulfonyl chloride (TPS) as condensing agents. Following condensation the protecting groups were removed with 9 *M* NH_4OH at 60 °C for 1 h (βCEP) and 80% acetic acid on a steam bath for 15 min (TBDMS). The yields of thymidine-3′ phosphate (Tp) from the two reactions were 100 and 98% respectively. The product Tp was unaffected by 5′-nucleotidase but was converted to thymidine with alkaline phosphatase. Compound **3***a* was similarly converted to thymidine-5′ phosphate (p.T.) which was hydrolized to thymidine with 5′-nucleotidase. These results not only confirm the identity of **2***a* and **3***a* but demonstrate the stability of the TBDMS group to phosphorylating conditions.

A major advantage of the TBDMS group is its compatibility with other commonly used protecting groups. This has greatly facilitated the preparation of nucleoside derivatives which are otherwise more difficult to obtain. For example the silyl group can be removed (see Table 3) with $(n\text{-Bu})_4NF$ in THF at room temperature for 30 min (5). These conditions do not affect acid labile groups (*e.g.* trityl) base labile protecting groups in general (*O*- or *N*-derivatives) and the β-benzoylpropionyl group in particular. On the other hand the hydrazine hydrate solution which removes *N*-acyl groups and the β-benzoylpropionyl group (7) has no

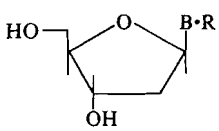
TABLE 3. Hydrolysis of the TBDMS group from **2***a*

Reagent used	Temperature (°C)	Time	% hydrolysis
$(n\text{-Bu})_4NF$ in THF	22	30 min	100
80% HOAC	Steam bath	15 min	100
0.5 *N* NaOH EtOH:H_2O (1:1)	22	24 h	80
9 *M* NH_4OH	60	1 h	6
15% NH_4OH in EtOH	22	2 h	0
H_2NNH_2: HOAc: Pyridine (1:6:24, (v/v/v)	22	24 h	0

affect on the TBDMS group. Thus treatment of **4***b* and **4***c* with benzoic anhydride and **4***d* with acetic anhydride followed by reaction with $(n\text{-Bu})_4NF$ leads to high yields of *N*-benzoyldeoxycytidine (**5***e*), *N*-benzoyldeoxyadenosine (**5***f*), and *N*-acetyldeoxyguanosine (**5***g*).



4*b–d* R = Benzoyl or Acetyl



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e; B = C; R = Bz
f; B = A; R = Bz
g; B = G; R = Ac

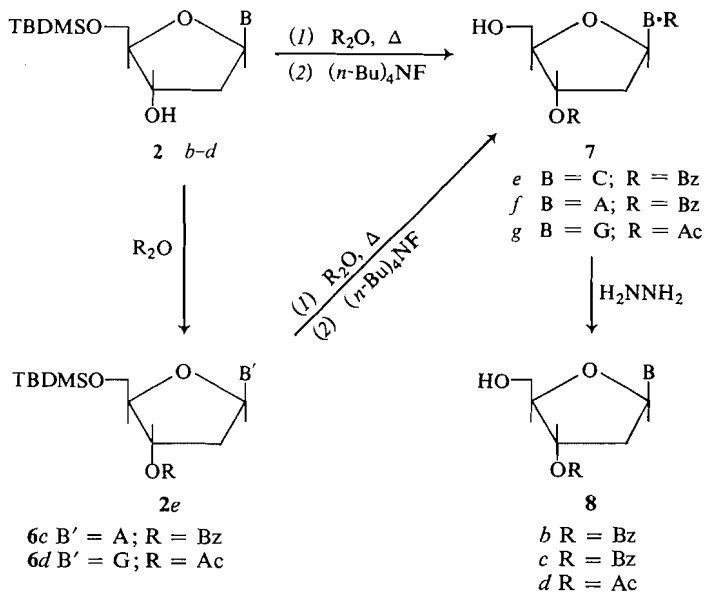
A very useful set of derivatives can easily be obtained as outlined in Scheme 3. Treatment of **2***b* with benzoyl chloride or by heating with benzoic anhydride followed by removal of the silyl group gives **7***e* in 80 and 82% yields respectively. **7***f* and *g* were obtained in a similar fashion in 93 and 86% respectively. We have found that **2***b* reacts with benzoic anhydride in pyridine at room temperature to produce 5′-TBDMS-*N*-benzoyldeoxycytidine (**2***c*) in good yield. This contrasts with **2***c* and *d* which react with benzoic anhydride and acetic anhydride respectively to give preferential *O*-acylation (**6***c* and *d*). The use of excess reagent and gentle heating causes *N*-acylation in these cases as well. Treatment of compounds **7** with hydrazine hydrate solution produces the 3′-acylated derivatives **8** in 90% yields.

Derivatives such as **7** are thus available from the starting nucleosides in two–three steps, two of the steps being ½ h reactions and overall yields being in the 50% range. Existing routes to these compounds usually require more and longer steps and lower overall yields. For example an existing route to **7***e* involves (8) tritylating deoxycytidine (62% yield) followed by benzoylation (71%) and removal of the trityl group (42%) to produce **7***e* in 18.5% overall yield. The acid hydrolsysis of the trityl group also causes hydrolysis of the *N*-glycoside bond in *N*-acylated nucleosides, a problem of particular importance with deoxyadenosine derivatives (9).

One of our goals in the nucleotide area is the development of methods which allow for the rapid identification of isomeric nucleoside and nucleotide derivatives even in very small amounts or from reaction mixtures. To this end we have been employing mass spectrometry (2). We have previously observed different fragmentation patterns from acyl groups of isomeric substituted nucleosides. We had hoped that the TBDMS group would increase the volatility of nucleoside derivatives and give clearly discernable fragmentation patterns from 3′- and 5′-isomeric derivatives. Most importantly, in addition to its use as an identification handle, the TBDMS group is a chemically useful protecting group. Table 4 shows some diagnostically important fragments from the mass spectra of the isomeric compounds **2** and **3**. Clearly fragmentation is significantly different between isomers. The ratio of peaks varies with temperature and with instrument conditions. A full analysis of the spectra of these silylated nucleosides will be presented shortly (10).

A possibility existed that the methyl groups on the TBDMS group of 3′- and 5′-isomers might show different resonances in the n.m.r. However, in the isomeric thymidines no useful distinction could be made with **2***a* having singlets δ ($CDCl_3$) of 0.14 ($(CH_3)_2Si$), 0.92 ($(CH_3)_3CSi$), and 1.91 p.p.m. (5-CH_3) and **3***a* showing singlets at 0.094 ($(CH_3)_2Si$), 0.89 ($(CH_3)_3CSi$), and 1.87 p.p.m. (5-CH_3). These differences are very slight and not considered by us to be of immediate practical use.

We have found most of the silylated nucleo-



SCHEME 3

TABLE 4. Some diagnostically important fragments from the mass spectra* of **2** and **3**

Compound	Temperature (°C)	Fragment, relative intensities M − 57	M − 75	M − 93	M − 155
2*a*	120	100	428	117	31
3*a*	120	100	0	0	455
2*b*	250	100	714	4	142
3*b*	200	100	83	0	800
2*c*	170	100	36	1	5.2
3*c*	100	100	0	0	224
2*d*	280	100	675	147	0
3*d*	280	100	12	0	112

*Spectra recorded on a Finnigan 1015 mass spectrometer.

sides to be crystalline compounds with sharp melting points. In a few cases initial attempts to induce crystallization have failed and the compounds were obtained as dry solids usually without clear melting points. As crystalline solids the silylated derivatives are stable for at least 10 months but we have detected loss of the protecting group on long standing in aqueous or alcohol solution. Gummy samples in which solvent has been incompletely removed also show deterioration on standing.

In conclusion we have found that TBDMSCL reacts selectively with the hydroxyl groups of nucleosides and preferentially with the 5′-hydroxyl group. Because of the compatibility of the TBDMS group with other acid and base labile protecting groups almost complete manipulation of protecting groups is now possible. The TBDMS group is stable to phosphorylation. The use of the TBDMS group is characterizing isomeric nucleosides and its use in synthesizing oligonucleotides in the ribose series will be described shortly.

Experimental

General Procedures

A detailed description of all our general procedures including solvent purification, chromatography techniques and reagents, phosphorylation procedures, enzyme assays, and analytical and instrumental methods and instruments has been given in detail in ref. 1*a* with the

following exceptions. Thin layer chromatography solvent 8:2 is used in this paper as an abbreviation for a solvent composed of chloroform: ethanol (8:2, v/v). Mass spectra were recorded on a Finnigan 1015 mass spectrometer. *tert*-Butyldimethylsilyl chloride, m.p. 121–125 °C, was prepared according to the method of Sommer and Taylor (4); it is also commercially available from Willow Brook Labs Inc. $(n\text{-Bu})_4$NF solution was prepared by neutralizing $(n\text{-Bu})_4$NOH with HF in methanol. After removal of the solvent, benzene–acetonitrile was evaporated from the residue several times. The residue was dried over P_2O_5 and made up to volume in THF (0.5 *M*). After treating a nucleoside derivative with the $(n\text{-Bu})_4$NF reagent, the solution was usually passed through a short column of Monday DSF-5 silica gel previously prepared on a t.l.c. plate. This procedure removed salts and greatly enhanced subsequent t.l.c. separations of products.

Synthesis of Compounds 2, 3, and 4 from 1

In all cases the nucleoside **1** (1 mmol) TBDMSCL and imidazole were added to 1 ml of DMF and the solution stirred at the desired temperature. At the end of the reaction the solution was applied to three t.l.c. plates developed first in ether and then in chloroform–ethanol (8:2). The separated bands were eluted with ethanol which on concentration yielded the products. Results are listed in Tables 1 and 2. Larger scale reactions were also carried out in a direct scale-up of this procedure. Specific changes for certain nucleosides are as follows.

Thymidine. T.l.c. plates were developed only in ether.

Deoxycytidine. T.l.c. plates were developed in THF. The starting nucleoside was deoxycytidine·HCl.

Deoxyguanosine. During the reactions most of the compound **4***d* precipitates and can be collected by filtration prior to chromatography.

N-Benzoyldeoxycytidine. T.l.c. plates developed only in ether.

N-Benzoyldeoxyadenosine. T.l.c. plates developed twice in ether.

$O^{5'}$-Pivaloyl-$O^{3'}$-TBDMS-thymidine

5′-Pivaloylthymidine (163 mg, 0.5 mmol), TBDMSCL (100 mg, 0.67 mmol), and imidazole (85 mg, 1.25 mol) were dissolved in DMF (0.5 ml) and stirred at 37 °C for 16 h. The solution was applied to two t.l.c. plates developed in ether. The single product band was eluted with ether, solvent was removed at reduced pressure and the residue was crystallized from ethanol to yield 200 mg (91%) of $O^{5'}$-pivaloyl-$O^{3'}$-TBDMS thymidine; m.p. 150–151 °C; R_f^1(Et_2O) 0.53; λ_{max} (EtOH) 208 and 266 nm; parent ion in mass spectrum at *m/e* 440.

Anal. Calcd. for $C_{21}H_{32}N_2O_6Si$: C, 57.24; H, 8.23; N, 6.36. Found: C, 57.11; H, 8.16; N, 6.42.

$O^{3'}$-TBDMS-thymidine (3a)

The above product (100 mg) was dissolved in 1 ml of THF and ethanol (1 ml) and 2*N* NaOH (1 ml) were added. The solution was stirred at room temperature for 6 h. The solution was neutralized with Dowex 50 W-X8 (pyridinium form), concentrated to a small volume and applied to a t.l.c. plate which was developed in ether. The product band and a thymidine band (at the origin) were eluted with ether and ethanol respectively. Thymidine was obtained in a 20% yield. The product **3***a* crystallized from ethanol–water, 55 mg (68%), m.p. 83–85 °C (see Table 2 for properties and analysis).

$O^{5'}$-Acetyl-N-benzoyldeoxycytidine, $O^{3'}$-Acetyl-N-benzoyldeoxycytidine, and $O^{3'}$,$O^{5'}$-Diacetyl-N-benzoyldeoxycytidine

Compound **1***e* (331 mg, 1 mmol) was dissolved in pyridine (5 ml) and acetic anhydride (0.1 ml) was added dropwise to the solution. After 1 h an additional 0.05 ml of acetic anhydride was added and the solution was stirred for a further 3 h. Ethanol (10 ml) was added and the solvents were removed at reduced pressure. The residue was applied to five t.l.c. plates which were developed three times in ethyl acetate. Beautiful separation occurred between the four bands which were eluted with ethanol. On concentration of each of the ethanol solutions the products crystallized from solution and were collected as follows.

The fastest moving material on t.l.c. was $O^{3'}$,$O^{5'}$-diacetyl-*N*-benzoyldeoxycytidine (54 mg, 13%): m.p. 193–194 °C; R_f (EtOAc) 0.36; *m/e* 415; λ_{max} (EtOH) 305 and 260 nm.

Anal. Calcd. for $C_{20}H_{21}N_3O_7$: C, 57.83; H, 5.09; N, 10.12. Found: C, 57.65; H, 5.01; N 10.05.

The next slowest product was $O^{3'}$-acetyl-*N*-benzoyldeoxycytidine (30 mg, 8%): m.p. 180–182 °C; R_f (EtOAc) 0.25; *m/e* 373; λ_{max} (EtOH) 305 and 260 nm.

Anal. Calcd. for $C_{18}H_{19}N_3O_6$: C, 57.90; H, 5.13; N, 11.25. Found: C, 57.85; H, 5.10; N, 11.28.

The second slowest product was $O^{5'}$-acetyl-*N*-benzoyldeoxycytidine (160 mg, 43%): m.p. 165–166 °C; R_f (EtOAc) 0.16; *m/e* 373; λ_{max} (EtOH) 305 and 260 nm.

Anal. Calcd. for $C_{18}H_{19}N_3O_6$: C, 57.90; H, 5.13; N, 11.25. Found: C, 57.82; H, 5.06; N, 11.32.

The slowest moving product on t.l.c. was **1***e* of which 83 mg (25%) was recovered.

$O^{3'}$-TBDMS-deoxycytidine (3b)

$O^{5'}$-Acetyl-*N*-benzoyldeoxycytidine (94 mg, 0.25 mmol), TBDMSCL (75 mg, 0.5 mmol), and imidazole (68 mg, 1 mmol) were added to DMF (0.5 ml) and the solution was stirred for 2 h. Thin layer chromatography showed complete conversion of starting material to a new product R_f (EtOAc) 0.65. The produce was separated from other reactants by passing it through a short column of silica gel (3 × 3.5 cm) with ether. The ether was evaporated and the residue was stirred with 50% NH_4OH in ethanol (4 ml) for 8 h. The solvents were removed at reduced pressure and the residue was dissolved in a solution containing pyridine (2.4 ml), acetic acid (0.6 ml), and hydrazine hydrate (0.1 ml). After 15 h the solvents were removed at reduced pressure and the residue was applied to a t.l.c. plate which was developed first in ether and then in 8:2. The product band was eluted with ethanol which on concentration yielded **3***b* (60%), see Table 2.

A second faster moving nucleoside band was obtained from the t.l.c. plate and was identified as $O^{5'}$-acetyl-$O^{3'}$-TBDMS-deoxycytidine: m.p. 120–122 °C (21%); $R_f^{8:2}$ 0.60; λ_{max} (EtOH) 274, 238 sh.

Anal. Calcd. for $C_{18}H_{29}N_5O_4Si$: C, 53.24; H, 7.62; N, 10.96. Found: C, 52.87; H, 7.55; N, 10.82.

Phosphorylations

Compounds **2***a* and **3***a* (50 mg in each experiment) were phosphorylated with β-cyanoethylphosphate using

either dicyclohexylcarbodiimide or triisopropylbenzenesulfonyl chloride as condensing agents using established procedures (1, 11, 12). The β-cyanoethyl group was removed using 9 *M* NH_4OH at 60 °C for 1 h. After removal of solvents the residue was heated on a steam bath for 15 min with 2 ml of 80% acetic acid. The solution was then concentrated at reduced pressure and applied to Whatman 3 mm paper developed in solvent A. Yields are described in the text.

Enzyme Studies

The procedures used for the 5′-nucleotidase and alkaline phosphatase have been described (1*a*). For each analysis 10 $O.D._{.267}$ units of nucleoside monophosphate were used and the product isolated by paper chromatography in solvent A (1*a*). The nucleoside monophosphate (Tp) obtained from **2***a* was resistant to 5′-nucleotidase but yielded thymidine when treated with alkaline phosphatase. From **3***a*, the nucleoside monophosphate (pT) gave thymidine when treated with either 5′-nucleotidase or alkaline phosphatase.

General Procedure for the Synthesis of **5***e–g*

Compound **4** (1 mmol) was dissolved in 5 ml of pyridine and an excess (5 mmol) of the anhydride (benzoic for **4***b* and **4***c* and acetic for **4***d*) was added. The solution was then stirred at room temperature for 24 h for **4***b*, heated on the steam bath for 3 h for **4***c*, and stirred at room temperature for 20 h followed by heating on a steam bath for 1 h for **4***d*. Solvents were removed at reduced pressure and by repeated evaporation of ethanol. $(n\text{-Bu})_4NF$ (2 ml) in THF was added and the solution stirred at room temperature for 30 min. The THF was evaporated and the residue dissolved in ethanol and applied to two t.l.c. plates developed in 8:2. The product band was eluted with ethanol and on concentration of the solvent compounds **5***e–g* were obtained in 94, 92, and 86% yields respectively. The products were identified by comparison to authentic samples prepared by the procedures of Khorana and co-workers (9). The compounds prepared by our procedure were identical (m.p., u.v., chromatography, and mass spectrometry) to those prepared by the Khorana procedure.

O^5′-TBDMS-O^3′,N-dibenzoyldeoxycytidine

Procedure (a)

Compound **2***b* (250 mg, 0.73 mmol) was dissolved in 5 ml of pyridine and 1 ml of benzoyl chloride was added. The solution was stirred at room temperature for 3 h, ethanol was added, and the solvents were removed at reduced pressure. The residue was dissolved in ethanol and applied to two t.l.c. plates developed in ether. The major product was eluted with ether and on evaporation of the solvent 350 mg (87%) of a white solid, m.p. 166–167 °C, were obtained: λ_{max} (EtOH) 305, 260, and 231 nm; *m/e* 549; R_f (Et_2O) 0.33, R_f (EtOAc) 0.41.

Anal. Calcd. for $C_{29}H_{35}N_3O_6Si$: C, 63.37; H, 6.41; N, 7.64. Found: C, 63.25; H, 6.40; N, 7.72.

Procedure (b)

Compound **2***b* (341 mg, 1 mmol) and benzoic anhydride (1.35 g, 6 mmol) were dissolved in pyridine (10 ml) and heated on a steam bath for 3 h. The solution was poured into ice and water (400 ml) and allowed to stand overnight. The solid was collected by filtration, dissolved in ethanol, and applied to two t.l.c. plates developed with ether. The product was eluted with ether, which on evaporation yielded 500 mg (91%) of 5′-TBDMS-3′,N-dibenzoyldeoxycytidine.

Procedure (c)

Compound **2***e* (100 mg) was heated with 1 mmol of benzoic anhydride in pyridine (2 ml) for 2 h on a steam bath and worked-up as in (*a*) above to give the desired product in 95% yield.

*O^5′-TBDMS-N-benzoyldeoxycytidine (***2***e)*

Compound **2***b* (220 mg, 0.64 mmol) and benzoic anhydride (226 mg, 1 mol) were dissolved in pyridine (2 ml) and stirred at room temperature for 3 h. The solution was applied directly to two t.l.c. plates which were developed first in ether and then in ethyl acetate. Three nucleoside bands were eluted and were identified as **2***b* (40 mg, 14%), **2***e* (160 mg, 56%), and 5′-TBDMS-O^3′-N-dibenzoyldeoxycytidine (50 mg, 14%).

*O^3′,N-Dibenzoyldeoxycytidine (***7***e)*

O^5′-TBDMS-O^3′,N-dibenzoyldeoxycytidine (550 mg, 1 mmol) was added to $(n\text{-Bu})_4NF$ in THF (4 ml) and the solution was stirred for 30 min. The solution was passed through a short (2 × 3 cm) column of silica gel which was thoroughly washed with THF. The THF solution was concentrated at reduced pressure and applied to four t.l.c. plates which were developed in ether. The product was eluted with ethanol and, on concentration of the solution, crystallized to give **7***e* (400 mg, 92%): m.p. 218–220 °C; λ_{max} (EtOH) 304, 260, and 232 nm; R_f (Et_2O) 0.20, R_f (EtOAc); *m/e* 435 (8).

*O^3′-Benzoyldeoxycytidine (***8***b)*

Compound **7***e* (305 mg, 0.7 mmol) was added to a solution containing pyridine (2.4 ml), acetic acid (0.6 ml), and hydrazine hydrate (0.1 ml) and the resulting solution was stirred for 20 h. Ethanol was added and the solvents were concentrated at reduced pressure and applied to two t.l.c. plates developed first in THF and then in ethanol. The product was eluted with ethanol which on complete evaporation left a white solid (**8***b*, 210 mg, 91%): m.p. dec. > 185 °C; λ_{max} EtOH 273 and 232 nm; R_f (THF) 0.07, R_f (8:2) 0.47; and *m/e* 331.

Anal. Calcd. for $C_{16}H_{17}N_3O_5$: C, 58.07; H, 5.16; N, 12.66. Found: C, 58.01; H, 5.02; N, 12.75.

*O^5′-TBDMS-O^3′-benzoyldeoxyadenosine (***6***c)*

Compound **2***c* (300 mg, 0.82 mmol) and benzoic anhydride (560 mg, 2.47 mmol) were added to pyridine (4 ml) and the solution was stirred at room temperature for 2.5 days. The solution was concentrated at reduced pressure and applied to three t.l.c. plates which were developed in ether yielding three nucleoside bands. These were eluted separately with ethanol and on concentration gave **2***c* (104 mg, 35%) and **6***c* (195 mg, 50%); m.p. 75–76 °C; λ_{max} (EtOH) 260 and 231 nm; R_f (Et_2O) 0.20, R_f (8:2) 0.80; *m/e* 469. Compound **6***c* will crystallize on slow evaporation of an ethanol – water solution.

Anal. Calcd. for $C_{23}H_{31}N_5O_4Si$: C, 58.82; H, 6.65; N, 14.91. Found: C, 59.01; H, 6.62; N, 14.83.

From the above reaction 48 mg of a material R_f (Et_2O) 0.25 was obtained and tentatively identified as O^5′-TBDMS-O^3′,N-dibenzoyldeoxyadenosine (λ_{max} (EtOH) 280 and 231 nm; *m/e* 516 (M − 57)).

*O^3′-N-Dibenzoyldeoxyadenosine (***7***f)*

(*a*) Compound **2***c* (730 mg, 2 mmol) and benzoic anhydride (1.8 g, 8 mmol) were dissolved in pyridine (10 ml)

and heated on a steam bath for 2 h. The solution was allowed to stir at room temperature for an additional 12 h whereupon ethanol (10 ml) was added and the solvents were removed at reduced pressure. The dry residue was dissolved in 5 ml of the $(n\text{-Bu})_4$NF solution and stirred for 30 min. The solution was filtered through a short (2 × 3 cm) column of silica gel which was washed with THF. The THF solution was concentrated at reduced pressure and applied to four t.l.c. plates which were developed twice in ethyl acetate. The product was eluted with ethanol and on concentration of the ethanol solution, **7***f* crystallized (850 mg, 93%): m.p. 112–114 °C; λ_{max} (EtOH) 280 and 233 nm; R_f (EtOAc) 0.26, R_f (THF) 0.77, R_f (8:2) 0.84; *m/e* 459.

Anal. Calcd. for $C_{24}H_{21}N_5O_5$: C, 62.74; H, 4.61; N, 15.24. Found: C, 62.66; H, 4.70; N, 15.15.

(*b*) The same general procedure as in (*a*) above gave a 92% yield of **7***f* starting from **6***c*.

*$O^{3'}$-Benzoyldeoxyadenosine (**8***c*)*

(*a*) Compound **7***f* (300 mg, 0.65 mmol) was treated with hydrazine in pyridine – acetic acid as above for **8***b*. The product crystallized from the reaction mixture and was collected by filtration, washed with ether, and dried to give **8***c* (220 mg, 96%). Compound **8***c* crystallized from ethanol: m.p. 264–265 °C; λ_{max}(EtOH) 259 and 232 nm; R_f (EtOAc) 0.12, R_f (THF) 0.63, R_f (8:2) 0.72; *m/e* 355.

Anal. Calcd. for $C_{17}H_{17}N_5O_4$: C, 57.46; H, 4.82; N, 19.71. Found: C, 57.40; H, 4.85; N, 19.65.

(*b*) Compound **8***c* was also obtained from **6***c* in 95% yield by treating **6***c* with $(n\text{-Bu})_4$NF solution in the usual manner.

*$O^{5'}$-TBDMS-$O^{3'}$-Acetyldeoxyguanosine (**6***d*)*

Compound **2***d* (285 mg, 0.75 mmol) and acetic anhydride (1 ml) were added to pyridine (5 ml) and stirred at room temperature for 2 days. The solution had become very viscous and a large amount of precipitate had formed which was collected by filtration and washed successively with pyridine, ether, and hexane. This solid material (190 mg) was pure **6***d*: m.p. dec. > 280 °C; λ_{max} (EtOH) 255 nm; R_f (THF) 0.10, R_f (8:2) 0.55; *m/e* 423. The filtrate and washings were applied to a t.l.c. plate developed in 8:2. An additional 87 mg of **6***d* was obtained.

Anal. Calcd. for $C_{18}H_{29}N_5O_5Si$: C, 51.04; H, 6.90; N, 16.53. Found: C, 49.88; H, 6.86; N, 16.45.

From the above t.l.c. plate 20 mg of a material having R_f (8:2) 0.71; λ_{max} (EtOH) 281, 261, and 255 nm; m.p. 188–189 °C; *m/e* 465, and without further characterization was assumed to be $O^{5'}$-TBDMS-$O^{3'}$,*N*-diacetyldeoxyguanosine.

*$O^{3'}$-N-Diacetyldeoxyguanosine (**7***g*)*

(*a*) The above experiment was repeated except that the solution was heated on a steam bath for 2 h, followed by the addition of ethanol (10 ml), and evaporation of the solvents. The residue was treated with 2 ml of the $(n\text{-Bu})_4$NF solution and following work-up as described for **7***f* (except that t.l.c. plates were developed in 8:2), the product **7***g* was obtained in 86% yield: m.p. 130–132 °C; λ_{max} (EtOH) 280, 260, and 255 nm; R_f (THF) 0.10, R_f (8:2) 0.50; and *m/e* 351.

Anal. Calcd. for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.88; N, 19.94. Found: C, 47.80; H, 4.82; N, 19.98.

(*b*) **7***g* was obtained in 90% yield by treating **2***g* with acetic anhydride in pyridine at room temperature and work-up as in (*a*) above.

*$O^{3'}$-Acetyldeoxyguanosine (**8***d*)*

(*a*) Compound **6***d* (85 mg, 0.2 mmol) was treated with 1 ml of the $(n\text{-Bu})_4$NF reagent in the usual manner. The product **8***d* was isolated by t.l.c. in 8:2 in 89% yield: m.p. slow dec. > 225 °C; λ_{max} (EtOH) 255 nm; R_f (THF) 0.00, R_f (8:2) 0.40; and *m/e* 309. This compound has been previously reported in the literature (13), m.p. dec. > 240 °C.

(*b*) Compound **8***d* was also obtained from **7***g* in 92% yield by treating **7***g* with the hydrazine in pyridine – acetic acid reagent.

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