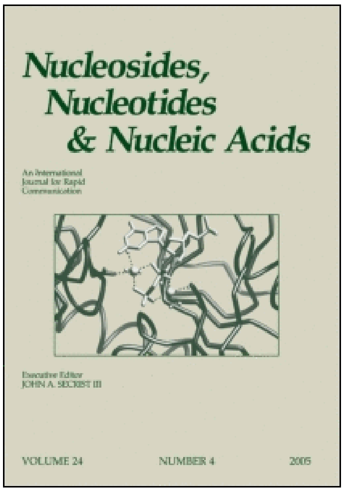

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SYNTHESIS AND ANTITUMOR ACTIVITY OF ACYL AND BENZYL TYPES OF PRODRUGS OF 2'-DEOXY-2'-METHYLIDENECYTIDINE †

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ABSTRACT: For the purpose of improvement of the *in vivo* antitumor activity of 2'-deoxy-2'-methylidenecytidine (DMDC, 1), we synthesized its various acyl and benzyl derivatives and evaluated them for their antitumor activity against P388 murine leukemia in mice. In terms of minimum effective dose (30% increase in life span), 5'-*O*-stearoyl DMDC showed two-fold higher antitumor activity than DMDC on a molar basis, when intraperitoneally (i.p.) administered to mice once a day. The antitumor activities of some other acyl derivatives were almost comparable to that of DMDC, while benzyl derivatives had no antitumor activity. Results on the hydrolysis of 5'-*O*-acyl derivatives by porcine liver esterase showed that at least these derivatives should not be resistant to enzymatic hydrolysis for exhibiting antitumor activity. After either an i.p. or oral dose of 3'-*O*-benzyl DMDC, very low concentrations of blood DMDC were seen compared with those after administration of DMDC, suggesting that the inactivity of benzyl derivatives as prodrugs was due to the minimal level of DMDC in circulation after administration.

INTRODUCTION

2'-Deoxy-2'-methylidenecytidine (DMDC, 1) was designed and synthesized as a new member of antitumor nucleosides.1) In contrast to 1-β-D-arabinofuranosylcytocine (ara-C), DMDC is resistant to cytidine deaminase, which inactivates ara-C to the

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† This paper is dedicated to the memory of the late Professor Tohru Ueda, who died on Sept. 19, 1990.

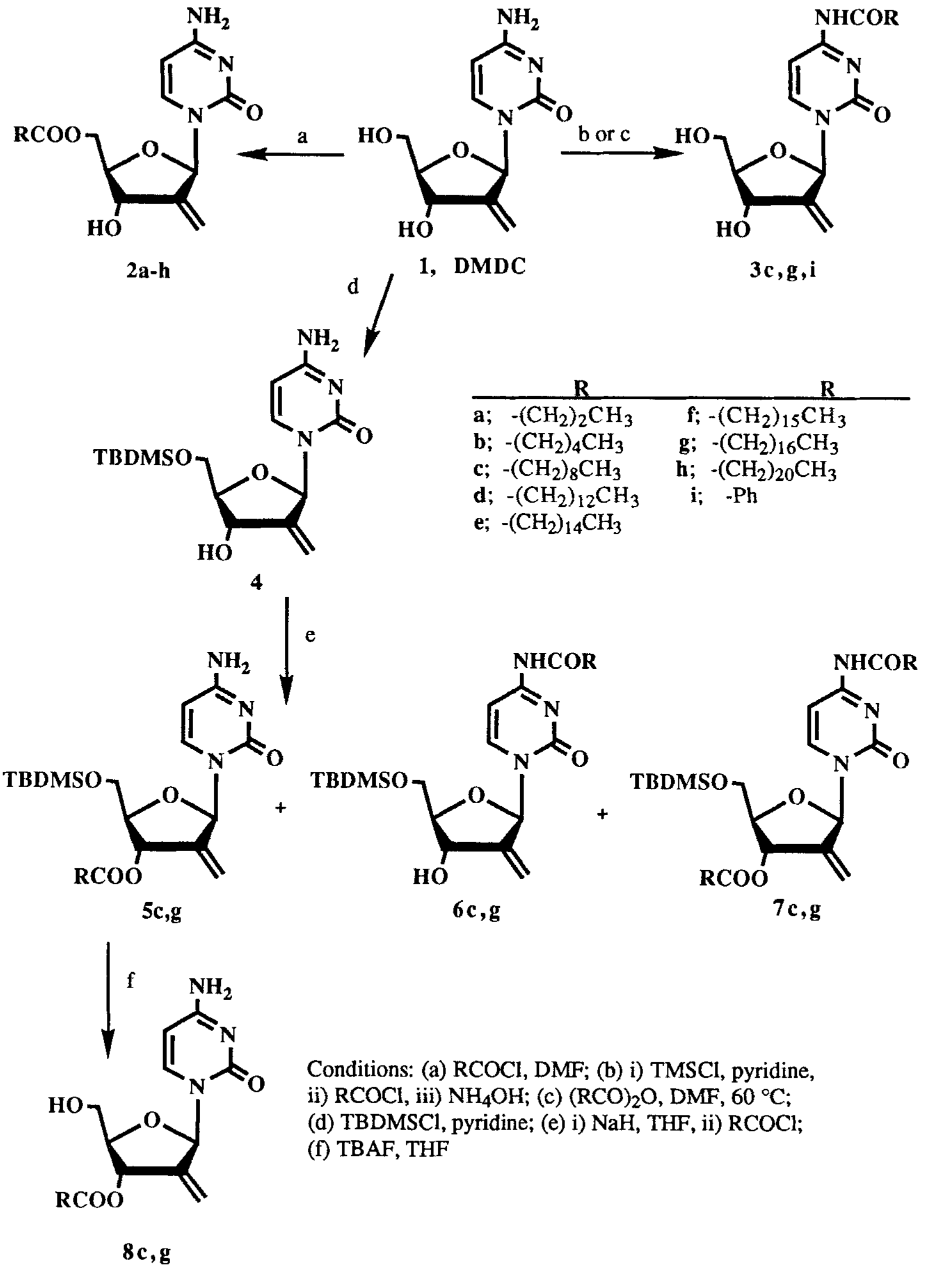
chemotherapeutically inactive ara-U, and is effective against not only against leukemia but also solid tumors such as the LX-1 human lung cancer xenograft and the SK-Mel-28 human melanoma xenograft, which are less sensitive or refractory to ara-C.2) Thus, DMDC is an interesting nucleoside from the viewpoint of anticancer chemotherapy.

For an antitumor nucleoside, the introduction of adequate substituents or protective groups into the parent compound could cause them to act as prodrugs and is expected to bring about the following effects: 1) increase of absorption by increasing drug lipophilicity, 2) depression of inactivation owing to anabolism of drugs, and 3) maintaining a high level of blood drug concentration through gradual formation of the active drug. These improvements could lead to reduction of the toxicity and/or increase therapeutic efficacy. Indeed, it has been reported that alteration in the physico-chemical properties of parent drugs consequently increased the chemotherapeutic effect in the case of ara-C, 5-fluorouracil, and 2'-deoxy-5-(trifluoromethyl)uridine (F_3Thd). 5'-*O*- And *N*^{4}-acyl derivatives of ara-C were reported to be more active than the parent ara-C.3,4) Recently, Yamashita *et al.* reported that the benzyl type derivatives of F_3Thd and 2'-deoxy-5-fluorouridine exhibited more potent antitumor activities.5) In this paper, we describe the synthesis of several acyl and benzyl derivatives of DMDC and their *in vivo* antitumor activity against the P388 murine leukemia. The relationship between enzymatic hydrolysis rate or blood concentration of DMDC and *in vivo* activity of these prodrugs is also discussed.

CHEMISTRY

It is necessary to determine the regioselective esterification of DMDC since there are three reactive centers in the molecule. The synthetic method for selective acylation at the 5'-hydroxyl group of ara-C·HCl described by Wechter et al.6) was applied to the synthesis of 5'-*O*-acyl derivatives of DMDC. The hydrochloric salt of DMDC was treated with stearoyl chloride in *N,N*-dimethylformamide (DMF) at room temperature to afford 5'-*O*-stearoyl DMDC (**2g**) in 38% yield. The H-5' signal of **2g** in the ^{1}H NMR spectrum appeared at lower magnetic field (δ 4.13-4.38) due to the ester bond at the 5' position than that of DMDC (δ 3.56-3.72). Similarly, 5'-*O*-butyryl, -octanoyl, -decanoyl, -myristoyl, -palmitoyl, -hexadecanoyl, and -behenoyl derivatives **2a-f**, **h** of DMDC were prepared in 30-59% yields.

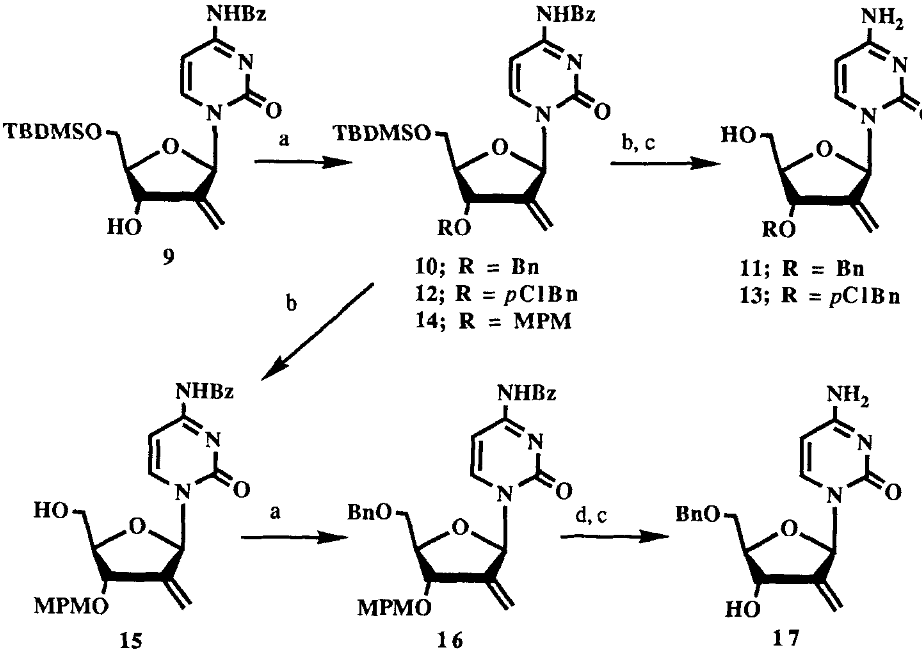
Introduction of an acyl group to the 4-amino group of DMDC was next examined by the one pot synthetic method of Jones et al.7) For the transient protection of hydroxyl groups of the sugar moiety, DMDC·HCl was treated with trimethylchlorosilane in pyridine, then acylation of the 4-amino group with stearoyl chloride was performed to give a fully protected DMDC, from which the desired *N*^{4}-stearoyl DMDC (**3g**) was obtained in 40%



R	R
a; $-(CH_2)_2CH_3$	f; $-(CH_2)_{15}CH_3$
b; $-(CH_2)_4CH_3$	g; $-(CH_2)_{16}CH_3$
c; $-(CH_2)_8CH_3$	h; $-(CH_2)_{20}CH_3$
d; $-(CH_2)_{12}CH_3$	i; -Ph
e; $-(CH_2)_{14}CH_3$	

Conditions: (a) RCOCl, DMF; (b) i) TMSCl, pyridine, ii) RCOCl, iii) NH_4OH; (c) $(RCO)_2O$, DMF, 60 °C; (d) TBDMSCl, pyridine; (e) i) NaH, THF, ii) RCOCl; (f) TBAF, THF

Scheme I



Conditions: (a) i) NaH, THF, ii) RBr or RCl; (b) TBAF; (c) NH_4OH, MeOH; (d) DDQ, CH_2Cl_2/H_2O;

Scheme II

yield after treatment with ammonia water to deblock the trimethylsilyl protection (method A). In a similar way, N^4-decanoyl DMDC (3c) was prepared. Whereas the UV spectrum of DMDC in MeOH showed an absorption maximum at 271 nm, compounds 3c and g showed a maximum at 300 nm due to N^4-acyl formation. The H-5 signal of 3g in the ^1H NMR spectrum appeared at lower magnetic field (δ 7.21) than that of DMDC (δ 5.72). Since this transient protection method gave the desired nucleoside in a rather low yield, we tried the reaction of DMDC with acid anhydride in DMF.$^{8)}$ From this method, we obtained N^4-decanoyl, -stearoyl, and -benzoyl derivatives 3c, g, i of DMDC in 79-87% yields (method B).

For the preparation of 3'-*O*-acyl derivatives of DMDC, protection of the 5'-hydroxyl group was necessary due to poor reactivity of the 3' position and steric hindrance. The choice of a protective group is, however, limited, since DMDC is somewhat unstable under acidic conditions due to the allylic systems in the molecule. We selected a *tert*-butyldimethylsilyl (TBDMS) group as a protection of the 5'-hydroxyl group. Reaction of

TABLE 1. Antitumor Activity of Derivatives of DMDC against P388 Leukemia

		%ILS Dose (mg/kg)				ILS_{30}	
Compound	Route	3	10	30	100	(mg/kg)	(μmol/kg)
DMDC (1)	i.p.	1.0	22.3	57.3		12	44
				59.1	93.9		
2a	i.p.	-1.9	17.5	58.3		15	49
2b	i.p.	20.4	29.1	35.9		16	44
2e	i.p.		13.0	29.6	64.3	30	63
2g	i.p.		28.7	70.4	126.1	10	20
2h	i.p.	19.4	2.9	16.5		>30	>53
3g	i.p.	-5.9	-6.7	0.8		>30	>59
8g	i.p.	-3.4	16.9	39.0		20	40
4	i.p.			10.7	28.6	>100	
4	p.o.				15.3	>100	
11	i.p.		7.4	6.5	0.9	>100	
11	p.o.				-11.0	>100	
13	i.p.		0.9	7.4	7.4	>100	
17	p.o.				-2.5	>100	

BDF_1 mice were (7 or 8 per group) inoculated with 10^6 P388 leukemia cells, and treated at the indicated dose daily from day 1 to 9.

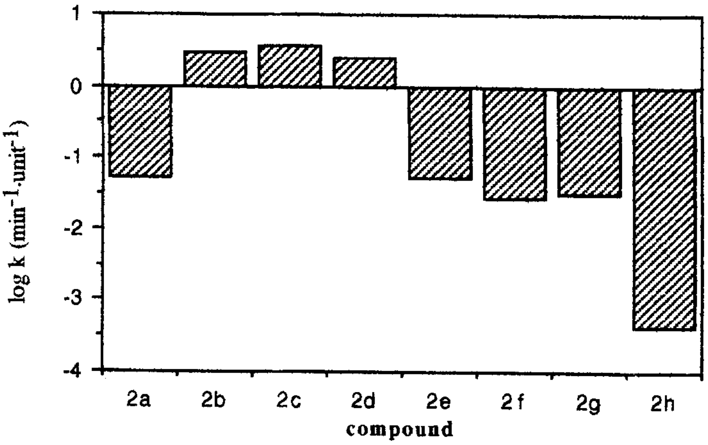


FIG. 1. Rates of hydrolysis of 5'-*O*-acyl derivatives of DMDC by porcine liver esterase. The esters were reacted with the enzyme, and amounts of DMDC produced by hydrolysis were determined by HPLC as described in text. Hydrolysis rates (k: $min^{-1} \cdot unit^{-1}$) were calculated according to the method of Nagai et al.[10)]

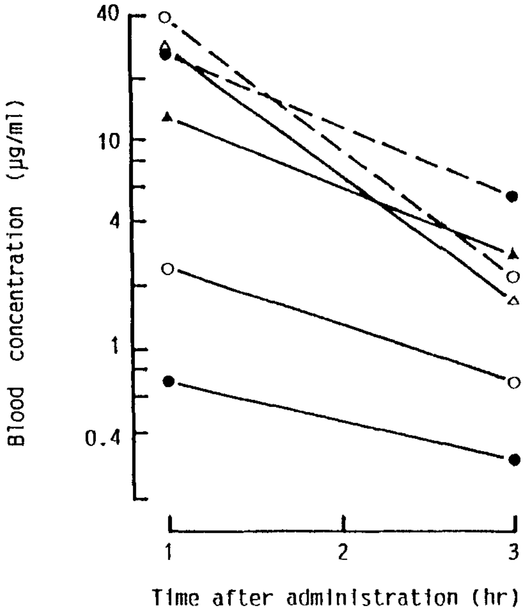


FIG. 2. Blood concentrations of DMDC and unchanged compound after i.p. or oral administrations of 3'-*O*-benzyl DMDC and DMDC. Means of blood concentrations of DMDC (solid lines) obtained from 4 mice were determined by HPLC 1 and 3 h after administration of DMDC (100 mg/kg, △ : i.p., and ▲ : oral) or 3'-*O*-benzyl DMDC (120 mg/kg, ○ : i.p., and ● : oral). Mean level of unchanged form of the prodrug was expressed by broken lines.

DMDC·HCl with *t*-butyldimethylchlorosilane (TBDMSCl) in pyridine afforded 5'-*O*-TBDMS DMDC (**4**) in 90% yield. Treatment of **4** with sodium hydride (NaH) in anhydrous tetrahydrofuran (THF) at room temperature, followed by addition of stearoyl chloride at -20 °C for 10 min gave the desired 3'-*O*-stearoyl DMDC (**5g**) along with N^4-stearoyl DMDC (**6g**) and N^4,3'-*O*-distearoyl DMDC (**7g**) as minor products. The ratio of these nucleosides **5g**, **6g**, and **7g** identified by ^{1}H-NMR spectroscopy of the mixture was 85 : 5 : 15. The formation of these by-products increased with increasing reaction time and reaction temperature. Deprotection of **5g** with tetrabutylammonium fluoride (TBAF) in THF afforded 3'-*O*-stearoyl DMDC (**8g**) in 30% yield from **4**. In a similar way, 3'-*O*-decanoyl DMDC (**8c**) was prepared.

Synthesis of 3'-*O*-benzyl derivatives was started from 5'-*O*-TBDMS-N^4-benzoyl DMDC (**9**), which had been obtained by treatment of N^4-benzoyl DMDC (**3i**) with TBDMSCl in DMF in the presence of imidazole. After treatment of **9** with NaH, benzyl bromide or *p*-chlorobenzyl bromide was added to the reaction mixture to give **10** or **12**, respectively. Removal of the silyl group with TBAF and then the benzoyl group with ammonia water gave the desired 3'-*O*-benzyl derivatives **11** and **13** in 44% and 57% yield from **9**, respectively.

By a similar method, 5'-*O*-benzyl DMDC (**17**) was synthesized. The *p*-methoxybenzyl group (MPM) was introduced at the 3' position of **9** for its protection, and then desilylation with TBAF gave N^4-benzoyl-3'-*O*-*p*-methoxybenzyl DMDC (**15**). After benzylation of the 5'-hydroxyl of **15**, 3'-MPM was removed with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) to give N^4-benzoyl-5'-*O*-benzyl DMDC. Removal of the benzoyl group with ammonia water in MeOH gave 5'-*O*-benzyl DMDC (**17**) in 20% yield from **9**.

ANTITUMOR ACTIVITY AND BIOLOGICAL BEHAVIORS

The antitumor activities of DMDC derivatives are given in TABLE 1. A marked increase in life span (ILS) was demonstrated by intraperitoneal (i.p.) administration of 5'-*O*-stearoyl DMDC (**2g**) at a dose of 30 or 100 mg/kg once a day. This compound showed two-fold higher antitumor activity than DMDC on a molar basis in terms of minimum effective dose (30% ILS). Some other acyl derivatives such as **2a**, **2b**, and **8g** showed almost equal antitumor effects to DMDC after i.p. treatment. On the other hand, N^4-stearoyl, 5'-*O*-behenoyl, and 5'-*O*-*t*-butyldimethylsilyl DMDC (**3g**, **2h**, and **4**) had little antitumor effect. Benzyl derivatives also had no antitumor activity. In a preliminary experiment, **2g** at doses of 50 and 100 mg/kg/day and **3g** at a dose of 50 mg/kg/day showed marked antitumor activity against L1210 leukemia in CDF_1 mice by i.p. administration for 5 days with about 200% ILS (data not shown).

To investigate a correlation of the antitumor activity and the susceptibility to enzymatic hydrolysis of 5'-*O*-acyl DMDC (**2**), we tested the rate of hydrolysis by porcine liver esterase (FIG. 1). It had been confirmed that DMDC was stable under the conditions for hydrolysis (76 units of esterase per ml at 37 °C for 60 min). According to the hydrolysis rate of these prodrugs, they were divided into three groups: slow [5'-*O*-behenoyl derivative **2h** ($k = 4.5\mathrm{x}10^{-4} \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$)], middle [5'-*O*-butyryl **2a** ($k = 5.6\mathrm{x}10^{-2} \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$), -palmitoyl **2e** ($k = 5.5\mathrm{x}10^{-2} \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$), -hexadecanoyl **2f** ($k = 2.9\mathrm{x}10^{-2} \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$), and -stearoyl **2g** ($k = 3.2\mathrm{x}10^{-2} \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$) derivatives], and rapid [5'-*O*-octanoyl **2b** ($k = 2.9 \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$), -decanoyl **2c** ($k = 3.8 \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$), and -myristoyl **2d** ($k = 2.6 \cdot \mathrm{min}^{-1} \cdot \mathrm{unit}^{-1}$) derivatives]. The rapidly hydrolyzed compounds **2b**-**d** were smoothly converted to DMDC, and **2b** showed the same antitumor activity as DMDC.

It was reported that 3'-*O*-benzyl F_3Thd was effectively activated to the active mother compound by drug-metabolizing enzyme(s) after absorption, and exhibited high antitumor activity.5) In contrast, 3'-*O*- and 5'-*O*-benzyl derivatives of DMDC did not show any activity (TABLE 1) by either i.p. or oral routes. Then, we determined blood concentrations of DMDC and its unchanged form after oral or i.p. administration of 3'-*O*-benzyl DMDC (**11**). As shown in FIG. 2, only minimal DMDC blood levels were demonstrated 1 and 3 h after i.p. and oral administrations of **11**, although relatively high level of unchanged form was seen. On the other hand, high blood levels were achieved at least 1 h after administrations of DMDC. This fact implies that unlike 3'-*O*-benzyl F_3Thd, **11** is well absorbed but little metabolized. This biological stability of **11** may relate to the inactivity of benzyl derivatives of DMDC.

EXPERIMENTAL

Melting points were measured on a Yamato MP-21 melting point apparatus and are uncorrected. Elemental analyses were carried out at a Yanaco MT-5. ^{1}H-NMR were recorded at 400 MHz on a JEOL GSX-400 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, dd = double doublet). All exchangeable protons were detected by addition of D_2O. UV absorption spectra were recorded on a Shimadzu UV-160A spectrophotometer. Mass spectra (MS) were measured on a JEOL AX-500 spectrometer (EI) and VG ZAB-HF spectrometer (FAB). TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was Merck Kieselgel 60 (70-230 mesh).

General method for the preparation of 5'-*O*-acyl-2'-deoxy-2'-methylidenecytidine (2a-h). Acid chloride (4.0 mmol) was added to a solution of the hydrochloride salt of 2'-deoxy-2'-methylidenecytidine[9] (**1**, 1.0 g, 3.6 mmol) in DMF (10 ml) in an ice bath and the reaction mixture was stirred overnight at room temperature. Then this acylation reaction was incomplete. After MeOH (5 ml) was added, the reaction mixture was stirred for 30 min and neutralized with saturated sodium hydrogen carbonate. The solvent was evaporated under reduced pressure, and the residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (10:1, v/v) to give the title compounds **2a-h**.

5'-*O*-Butyryl-2'-deoxy-2'-methylidenecytidine (2a). The reaction of **1** with butyryl chloride (0.42 ml) gave **2a** (0.44 g, 39%) as a foam. MS *m/z* (FAB): 310 $[(M+H)^+]$. UV λ_{max} (MeOH) 271 nm (ε 7,000), 241 nm (ε 7,100); UV λ_{min} (MeOH) 259 nm (ε 6,500). ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, Me), 1.54 (2H, m, methylene-H), 2.31 (2H, t, $-CH_2-CO-$), 3.77 (1H, m, H-4'), 4.14-4.39 (2H, m, H-5'a,b), 4.47 (1H, m, H-3'), 5.17 (1H, t, H-2''a), 5.35 (1H, t, H-2''b), 5.73 (1H, d, 3'-OH), 5.82 (1H, d, J = 7.8 Hz, H-5), 6.52 (1H, d, J = 1.5 Hz, H-1'), 7.24 (1H, br s, NH), 7.28

(1H, br s, NH), 7.38 (1H, d, *J* = 7.8 Hz, H-6). *Anal.* Calcd for $C_{14}H_{19}N_3O_5{\cdot}2/3H_2O$: C, 52.33; H, 6.38; N, 13.08. Found: C, 52.41; H, 6.30; N, 12.89.

5'-*O*-Octanoyl-2'-deoxy-2'-methylidenecytidine (2b). The reaction of **1** with octanoyl chloride (0.68 ml) gave **2b** (0.56 g, 42%) as a foam. MS *m/z* (FAB): 366 $[(M+H)^+]$. UV λ_{max} (MeOH) 272 nm (ε 6,900), 240 nm (ε 7,000); UV λ_{min} (MeOH) 259 nm (ε 6,400). ^1H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.53 (10H, m, methylene-H), 2.31 (2H, t, $-CH_2-CO-$), 3.76 (1H, m, H-4'), 4.14-4.38 (2H, m, H-5'a,b), 4.46 (1H, m, H-3'), 5.16 (1H, t, H-2"a), 5.34 (1H, t, H-2"b), 5.73 (1H, d, 3'-OH), 5.82 (1H, d, H-5), 6.52 (1H, d, *J* = 2.0 Hz, H-1'), 7.24 (1H, br s, NH), 7.30 (1H, br s, NH), 7.38 (1H, d, H-6). *Anal.* Calcd for $C_{18}H_{27}N_3O_5{\cdot}H_2O$: C, 56.38; H, 7.62; N, 10.96. Found: C, 56.04; H, 7.45; N, 10.78.

5'-*O*-Decanoyl-2'-deoxy-2'-methylidenecytidine (2c). The reaction of **1** with decanoyl chloride (0.83 ml) gave **2c** (0.84 g, 59%) as a foam. MS *m/z* (FAB): 394 $[(M+H)^+]$. UV λ_{max} (MeOH) 273 nm (ε 6,400); UV λ_{min} (MeOH) 254 nm (ε 5,500). ^1H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.52 (14H, m, methylene-H), 2.31 (2H, t, $-CH_2-CO-$), 3.80 (1H, m, H-4'), 4.14-4.38 (2H, m, H-5'a,b), 4.48 (1H, m, H-3'), 5.26 (1H, t, H-2"a), 5.39 (1H, t, H-2"b), 5.90 (1H, d, *J* = 7.8 Hz, H-5), 6.49 (1H, d, *J* = 1.4 Hz, H-1'), 7.56 (1H, d, *J* = 7.8 Hz, H-6), 7.85 (1H, br s, NH), 8.32 (1H, br s, NH). *Anal.* Calcd for $C_{20}H_{31}N_3O_5{\cdot}H_2O$: C, 58.38; H, 8.08; N, 10.21. Found: C, 58.12; H, 8.00; N, 9.85.

5'-*O*-Myristoyl-2'-deoxy-2'-methylidenecytidine (2d). The reaction of **1** with myristoyl chloride (1.1 ml) gave **2d** (0.75 g, 46%, crystallized from AcOEt); mp 107-111 °C. MS *m/z* (FAB): 450 $[(M+H)^+]$. UV λ_{max} (MeOH) 270 nm (ε 7,500), 241 nm (ε 7,700); UV λ_{min} (MeOH) 258 nm (ε 7,100). ^1H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.52 (22H, m, methylene-H), 2.31 (2H, t, $-CH_2-CO-$), 3.77 (1H, m, H-4'), 4.14-4.38 (2H, m, H-5'a,b), 4.46 (1H, m, H-3'), 5.16 (1H, t, H-2"a), 5.34 (1H, t, H-2"b), 5.73 (1H, d, *J* = 7.3 Hz, H-5), 5.83 (1H, d, 3'-OH), 6.52 (1H, d, *J* = 1.4 Hz, H-1'), 7.22 (1H, br s, NH), 7.28 (1H, br s, NH), 7.38 (1H, d, *J* = 7.3 Hz, H-6). *Anal.* Calcd for $C_{24}H_{39}N_3O_5$: C, 64.12; H, 8.74; N, 9.35. Found: C, 63.97; H, 8.66; N, 9.01.

5'-*O*-Palmitoyl-2'-deoxy-2'-methylidenecytidine (2e). The reaction of **1** with palmitoyl chloride (1.1 g) gave **2e** (0.82 g, 47%, crystallized from AcOEt-ether); mp 106-112 °C. MS *m/z* (FAB): 478 $[(M+H)^+]$. UV λ_{max} (MeOH) 271 nm (ε 7,700), 241 nm (ε 7,800); UV λ_{min} (MeOH) 258 nm (ε 7,100). ^1H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.52 (26H, m, methylene-H), 2.31 (2H, t, $-CH_2-CO-$), 3.76 (1H, m, H-4'), 4.14-4.38 (2H, m, H-5'a,b), 4.46 (1H, m, H-3'), 5.16 (1H, t, H-2"a), 5.34 (1H, t, H-2"b), 5.72 (1H, d, 3'-OH), 5.80 (1H, d, H-5), 6.52 (1H, d, *J* = 2.0 Hz, H-1'), 7.24

(1H, br s, NH), 7.26 (1H, br s, NH), 7.38 (1H, d, H-6). *Anal.* Calcd for $C_{26}H_{43}N_3O_5$: C, 65.38; H, 9.07; N, 8.80. Found: C, 65.12; H, 9.06; N, 8.67.

5'-*O*-Hexadecanoyl-2'-deoxy-2'-methylidenecytidine (2f). The reaction of **1** with hexadecanoyl chloride (1.2 g) gave **2f** (0.77 g, 43%, crystallized from AcOEt); mp 110-113 °C. MS *m/z* (FAB): 492 [$(M+H)^+$]. UV λ_{max} (MeOH) 270 nm (ε 7,900), 241 nm (ε 8,100); UV λ_{min} (MeOH) 258 nm (ε 7,400). ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.53 (28H, m, methylene-H), 2.31 (2H, t, -CH_2-CO-), 3.76 (1H, m, H-4'), 4.13-4.37 (2H, m, H-5'a,b), 4.59 (1H, m, H-3'), 5.16 (1H, t, H-2"a), 5.33 (1H, t, H-2"b), 5.72 (1H, d, *J* = 7.3 Hz, H-5), 5.79 (1H, d, 3'-OH), 6.51 (1H, d, *J* = 1.9 Hz, H-1'), 7.23 (1H, br s, NH), 7.26 (1H, br s, NH), 7.37 (1H, d, *J* = 7.3 Hz, H-6). *Anal.* Calcd for $C_{27}H_{45}N_3O_5$: C,65.96; H, 9.23; N, 8.54. Found: C, 65.74; H, 9.24; N, 8.23.

5'-*O*-Stearoyl-2'-deoxy-2'-methylidenecytidine (2g). The reaction of **1** with stearoyl chloride (1.3 ml) gave **2g** (0.69 g, 38%, crystallized from AcOEt); mp 104-105 °C. MS *m/z* (FAB): 506 [$(M+H)^+$]. UV λ_{max} (MeOH) 270 nm (ε 7,800), 241 nm (ε 8,300); UV λ_{min} (MeOH) 259 nm (ε 7,500). ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.52 (30H, m, methylene-H), 2.31 (2H, t, -CH_2-CO-), 3.76 (1H, m, H-4'), 4.13-4.38 (2H, m, H-5'a,b), 4.46 (1H, m, H-3'), 5.16 (1H, t, H-2"a), 5.34 (1H, t, H-2"b), 5.72 (1H, d, 3'-OH), 5.80 (1H, d, *J* = 6.3 Hz, H-5), 6.52 (1H, d, *J* = 2.0 Hz, H-1'), 7.23 (1H, br s, NH), 7.27 (1H, br s, NH), 7.37 (1H, d, *J* = 6.3 Hz, H-6). *Anal.* Calcd for $C_{28}H_{47}N_3O_5$: C, 66.50; H, 9.37; N, 8.31. Found: C, 66.32; H, 9.36; N, 8.17.

5'-*O*-Behenoyl-2'-deoxy-2'-methylidenecytidine (2h). The reaction of **1** with behenoyl chloride (1.4 g) gave **2h** (0.61 g, 30%, crystallized from AcOEt); mp 98-102 °C. MS *m/z* (FAB): 562 [$(M+H)^+$]. UV λ_{max} (MeOH) 272 nm (ε 7,500); UV λ_{min} (MeOH) 256 nm (ε 6,700). ^{1}H NMR(DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.52 (38H, m, methylene-H), 2.31 (2H, t, -CH_2-CO-), 3.76 (1H, m, H-4'), 4.13-4.37 (2H, m, H-5'a,b), 4.46 (1H, m, H-3'), 5.16 (1H, t, H-2"a), 5.34 (1H, t, H-2"b), 5.72 (1H, d, 3'-OH), 5.80 (1H, d, *J* = 6.3 Hz, H-5), 6.52 (1H, d, *J* = 1.5 Hz, H-1'), 7.23 (1H, br s, NH), 7.27 (1H, br s, NH), 7.37 (1H, d, *J* = 6.3 Hz, H-6). *Anal.* Calcd for $C_{32}H_{55}N_3O_5$: C, 68.41; H, 9.87; N, 7.48. Found: C, 68.20; H, 9.94; N, 7.33.

General method A for the preparation of *N*^{4}-acyl-2'-deoxy-2'-methylidenecytidine (3c and g). Trimethylchlorosilane (0.30 ml, 2.4 mmol) was added to a solution of the hydrochloride salt of 2'-deoxy-2'-methylidenecytidine (**1**, 276 mg, 1.0 mmol) in dry pyridine (5 ml) at room temperature and the solution was stirred for 1 h. Acyl chloride (5.0 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. The mixture was then cooled in an ice bath, and water (2ml) was added. After 5 min, 28% aqueous ammonia (2 ml) was added, and the mixture was stirred at room temperature for 15 min. The mixture was evaporated *in vacuo* to near dryness. The residue was dissolved in $CHCl_3$, and the solution was washed once with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a silica gel column and eluted with $CHCl_3$-MeOH (10:1, v/v) to give title compounds **3c** and **g**.

***N*⁴-Decanoyl-2'-deoxy-2'-methylidenecytidine (3c).** The reaction of **1** with decanoyl chloride (1.0 ml) gave **3c** (0.21 g, 53%, crystallized from EtOH); mp 136-137 °C. MS *m/z*: 393 (M^+). UV λ_{max} (MeOH) 300 nm (ε 7,800), 250 nm (ε 17,400); UV λ_{min} (MeOH) 278 nm (ε 4,800). ^1H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.24-1.55 (14H, m, methylene-H), 2.38 (2H, t, -CH_2-CO-), 3.61-3.74 (3H, m, H-4',5'a,b), 4.52 (1H, m, H-3'), 5.04 (1H, t, 5'-OH), 5.32 (1H, t, H-2"a), 5.35 (1H, t, H-2"b), 5.69 (1H, d, 3'-OH), 6.55 (1H, d, *J* = 1.5 Hz, H-1'), 7.21 (1H, d, *J* = 7.8 Hz, H-5), 8.10 (1H, d, *J* = 7.8 Hz, H-6), 10.87 (1H, s, NH). *Anal.* Calcd for $C_{20}H_{31}N_3O_5\cdot 1/4H_2O$: C, 60.36; H, 7.98; N, 10.56. Found: C, 60.47; H, 7.94; N, 10.50.

***N*⁴-Stearoyl-2'-deoxy-2'-methylidenecytidine (3g).** The reaction of **1** with stearoyl chloride (1.7 ml) gave **3g** (0.20 g, 40%, crystallized from EtOH); mp 116-120 °C. MS *m/z* (FAB): 506 [$(M+H)^+$]. UV λ_{max} (MeOH) 300 nm (ε 7,300), 250 nm (ε 16,200); UV λ_{min} (MeOH) 276 nm (ε 4,300), 229 nm (ε 5,500). ^1H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.54 (30H, m, methylene-H), 2.38 (2H, t, -CH_2-CO-), 3.60-3.73 (3H, m, H-4',5'a,b), 4.52 (1H, m, H-3'), 5.03 (1H, t, 5'-OH), 5.32 (1H, t, H-2"a), 5.35 (1H, t, H-2"b), 5.68 (1H, d, 3'-OH), 6.55 (1H, d, *J* = 1.5 Hz, H-1'), 7.21 (1H, d, *J* = 7.3 Hz, H-5), 8.10 (1H, d, *J* = 7.3 Hz, H-6), 10.87 (1H, s, NH). *Anal.* Calcd for $C_{28}H_{47}N_3O_5\cdot 1/2H_2O$: C, 65.34; H, 9.40; N, 8.16. Found: C, 65.57; H, 9.48; N, 7.79.

General method B for the preparation of *N*⁴-acyl-2'-deoxy-2'-methylidenecytidine (3c, g, and i). A mixture of 2'-deoxy-2'-methylidenecytidine hydrate (DMDC·$2H_2O$, **1**, 275 mg, 1.0 mmol) and acid anhydride (1.2 mmol) in DMF (20 ml) was heated at 60 °C for 3 h. The solvent was evaporated *in vacuo* to dryness. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (10:1, v/v) to give title compounds **3c**, **g**, and **i**.

***N*⁴-Decanoyl-2'-deoxy-2'-methylidenecytidine (3c).** The reaction of **1** with decanoic anhydride (0.39 g) gave **3c** (0.33 g, 84%, crystallized from EtOH); mp 136-137 °C. *Anal.* Calcd for $C_{20}H_{31}N_3O_5\cdot 1/4H_2O$: C, 60.36; H, 7.98; N, 10.56. Found: C, 60.20; H, 7.79; N, 10.43. UV, ^1H NMR, and MS spectroscopy were identical with those of material prepared by method A.

N^4-Stearoyl-2'-deoxy-2'-methylidenecytidine (3g). The reaction of **1** with stearic anhydride (0.66 g) gave **3g** (0.40 g, 79%, crystallized from EtOH); mp 116-120 °C. *Anal.* Calcd for $C_{28}H_{47}N_3O_5 \cdot 1/4H_2O$: C, 65.92; H, 9.38; N, 8.24. Found; C, 66.03; H, 9.40; N, 7.96. UV, ^{1}H NMR, and MS spectroscopy were identical with those of material prepared by method A.

N^4-Benzoyl-2'-deoxy-2'-methylidenecytidine (3i). After the reaction of **1** with benzoic anhydride (0.25 g), the solvent was removed *in vacuo* to dryness. The solid residue was washed with EtOH and collected to give **3i** (0.30 g, 87%); mp >300 °C. *Anal.* Calcd for $C_{17}H_{17}N_3O_3$: C, 59.47; H, 4.99; N, 12.24. Found: C, 59.27; H, 5.02; N, 12.21. ^{1}H NMR and MS spectroscopy were identical with those of material prepared according to the literature.[9)]

5'-*O-tert*-Butyldimethylsilyl-2'-deoxy-2'-methylidenecytidine (4). *tert*-Butyldimethylchlorosilane (TBDMSCl, 0.78 g, 5.2 mmol) was added to a solution of the hydrochloride salt of 2'-deoxy-2'-methylidenecytidine (**1**, 1.3 g, 4.7 mmol) in dry pyridine (10 ml). The mixture was stirred at room temperature for 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in $CHCl_3$, and the solution was washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (10:1, v/v) to give **4** (1.5 g, 90%, crystallized from benzene); mp 127-131 °C. MS *m/z*: 353 (M^+). ^{1}H NMR (DMSO-d_6) δ 0.05 (3H, s, Si-Me), 0.06 (3H, s, Si-Me), 0.88 (9H, s, Si-*t*-Bu), 3.69 (1H, m, H-4'), 3.76-3.92 (2H, m, H-5'a,b), 4.47 (1H, d, H-3'), 5.29 (1H, t, H-2"a), 5.37 (1H, t, H-2"b), 5.74 (1H, br s, 3'-OH), 5.90 (1H, d, H-5), 6.51 (1H, d, J = 1.5 Hz, H-1'), 7.67 (1H, d, H-6), 7.91 (1H, br s, NH), 8.41 (1H, br s, NH). *Anal.* Calcd for $C_{16}H_{27}N_3O_4Si \cdot 1/4H_2O$: C, 53.68; H, 7.74; N, 11.74. Found: C, 53.64; H, 7.70, N, 11.54.

5'-*O-tert*-Butyldimethylsilyl-3'-*O*-decanoyl-2'-deoxy-2'-methylidenecytidine (5c). NaH (60% in mineral oil, 0.04 g, 3.6 equiv.) was added to a solution of compound **4** (1.0 g, 2.8 mmol) in THF (35 ml). The mixture was stirred at room temperature for 30 min, then cooled to -20 °C, and decanoyl chloride (0.58 ml, 2.8 mmol) was added dropwise. After 10 min, *p*-toluenesulfonic acid (*p*-TsOH, 1.4 g, 7.3 mmol) was added for neutralization, and the mixture was raised to 0 °C. Water was added to the reaction mixture, and the mixture was quickly extracted with $CHCl_3$. The organic layer was dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was separated on a silica gel column chromatography by eluting with $CHCl_3$-MeOH (100:1, v/v) to give 5'-*O-tert*-butyldimethylsilyl-N^4,3'-*O*-didecanoyl-2'-deoxy-2'-methylidenecytidine (**7c**, *Rf* = 0.80, $CHCl_3$: EtOH = 10:1, v/v) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.11 (6H, s, Si-Me), 0.86-0.94 (15H, m, Si-*t*-Bu and Me x 2), 1,28-1.69

(28H, m, methylene-H), 2.34-2.48 (4H, m, -CH_2-CO- x 2), 3.87-3.99 (2H, m, H-5'a,b), 4.12 (1H, m, H-4'), 5.42 (1H, t, H-2"a), 5.47 (1H, t, H-2"b), 5.78 (1H, m, H-3'), 6.91 (1H, d, J = 1.4 Hz, H-1'), 7.41 (1H, d, J = 7.8 Hz, H-5), 8.20 (1H, d, J = 7.8 Hz, H-6), 9.6 (1H, br s, NH). Continuous elution of the column with $CHCl_3$-MeOH (50:1, v/v) afforded a mixture of 5'-*O-tert*-butyldimethylsilyl-N^4-decanoyl-2'-deoxy-2'-methylidenecytidine (**6c**, *Rf* = 0.58) and 5'-*O-tert*-butyldimethylsilyl-3'-*O*-decanoyl-2'-deoxy-2'-methylidenecytidine (**5c**, *Rf* = 0.43) as an oil. ^{1}NMR and MS spectroscopy were obtained from the spectrum of the mixture. Data for **5c**; MS *m/z*: 507 (M^+). ^{1}H NMR ($CDCl_3$) δ 0.09 (3H, s, Si-Me), 0.10 (3H, s, Si-Me), 0.88 (3H, t, Me), 0.91 (9H, s, Si-*t*-Bu), 1.27-1.65 (14H, m, methylene-H), 2.35 (2H, t, -CH_2-CO-), 3.85-3.91 (2H, m, H-5'a,b), 4.05 (1H, m, H-4'), 5.31 (1H, t, H-2"a), 5.45 (1H, t, H-2"b), 5.68 (1H, d, J = 7.3 Hz, H-5), 5.74 (1H, m, H-3'), 6.91 (1H, d, J = 1.5 Hz, H-1'), 7.72 (1H, d, J = 7.3 Hz, H-6).

3'-*O*-Decanoyl-2'-deoxy-2'-methylidenecytidine (8c). A solution of the mixture of **5c** and **6c** in THF (10 ml) was treated with TBAF (1M THF solution, 1.7 ml, 1.7 mmol) at room temperature for 1 h. The THF was removed *in vacuo*. The residue was separated on a silica gel column chromatography by eluting with $CHCl_3$-MeOH (10:1, v/v) to give **8c** (0.44 g, 40% from **4**, crystallized from AcOEt); mp 90-94 °C. UV λ_{max} (MeOH) 270 nm (ε 7,700), 241 nm (ε 7,900); UV λ_{min} (MeOH) 258 nm (ε 7,300). ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, Me), 1.24-1.54 (14H, m, methylene-H), 2.36 (2H, t, CH_2-CO-), 3.59-3.62 (2H, m, H-5'a,b), 3.94 (1H, m, H-4'), 5.07 (1H, t, 5'-OH), 5.14 (1H, t, H-2"a), 5.39 (1H, t, H-2"b), 5.70 (1H, m, H-3'), 5.74 (1H, d, J = 7.3 Hz, H-5), 6.60 (1H, d, J = 2.0 Hz, H-1'), 7.26 (1H, br s, NH), 7.31 (1H, br s, NH), 7.57 (1H, d, J = 7.3 Hz, H-6). *Anal.* Calcd for $C_{20}H_{31}N_3O_5$: C, 61.05; H, 7.94; N, 10.68. Found: C, 61.30; H, 8.01; N, 10.48.

5'-*O-tert*-Butyldimethylsilyl-3'-*O*-stearoyl-2'-deoxy-2'-methylidenecytidine (5g). In a manner similar to that described for **5c**, compound **4** (1.0 g, 2.8 mmol) was treated with stearoyl chloride (0.94 ml, 2.8 mmol). The mixture was separated on a silica gel column chromatography by eluting with $CHCl_3$-MeOH (100:1, v/v) to give 5'-*O-t*-butyldimethylsilyl-N^4,3'-*O*-distearoyl-2'-deoxy-2'-methylidenecytidine (**7g**, *Rf* = 0.85, $CHCl_3$: EtOH = 10:1, v/v) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.11 (6H, s, Si-Me), 0.86-0.95 (15H, m, Si-*t*-Bu and Me x 2), 1.25-1.69 (60H, m, methylene-H), 2.34-2.44 (4H, m, -CH_2-CO- x 2), 3.88-3.99 (2H, m, H-5'a,b), 4.11 (1H, m, H-4'), 5.40 (1H, t, H-2"a), 5.47 (1H, t, H-2"b), 5.77 (1H, m, H-3'), 6.92 (1H, d, J = 1.5 Hz, H-1'), 7.37 (1H, d, J = 7.8 Hz, H-5), 8.19 (1H, d, J = 7.8 Hz, H-6), 8.53 (1H, br s, NH). Continuous elution of the column with $CHCl_3$-MeOH (25:1, v/v) afforded a mixture of 5'-*O-t*-butyldimethylsilyl-N^4-stearoyl-2'-deoxy-2'-methylidenecytidine (**6g**, *Rf* = 0.58) and

5'-*O*-*t*-butyldimethylsilyl-3'-*O*-stearoyl-2'-deoxy-2'-methylidenecytidine (**5g**, *Rf* = 0.43) as an oil. ^{1}NMR and MS spectroscopy were obtained from the spectrum of the mixture. Data for **5g**; MS *m/z*: 619 (M$^+$). ^{1}H NMR (CDCl$_3$) δ 0.09 (3H, s, Si-Me), 0.10 (3H, s, Si-Me), 0.88 (3H, t, Me), 0.91 (9H, s, Si-*t*-Bu), 1.25-1.63 (30H, m, methylene-H), 2.34 (2H, t, -CH$_2$-CO-), 3.88-3.91 (2H, m, H-5'a,b), 4.05 (1H, m, H-4'), 5.31 (1H, t, H-2"a), 5.45 (1H, t, H-2"b), 5.68 (1H, d, *J* = 7.3 Hz, H-5), 5.74 (1H, m, H-3'), 6.91 (1H, d, *J* = 1.4 Hz, H-1'), 7.72 (1H, d, *J* = 7.3 Hz, H-6).

3'-*O*-Stearoyl-2'-deoxy-2'-methylidenecytidine (8g). In a manner similar to that described for **8c**, desilylation of the mixture of **5g** and **6g** with TBAF (1 M THF solution, 1.7 ml, 1.7 mmol) gave **8g** (0.42 g, 30% from **4**, crystallized from AcOEt); mp 111-114 °C. MS *m/z* (FAB): 506 [(M+H)$^+$]. UV λ_{max} (MeOH) 270 nm (ε 8,000), 240 nm (ε 8,500); UV λ_{min} (MeOH) 258 nm (ε 7,700). ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, Me), 1.23-1.56 (30H, m, methylene-H), 2.36 (2H, t, -CH$_2$-CO-), 3.60-3.62 (2H, m, H-5'a,b), 3.93 (1H, m, H-4'), 5.06 (1H, t, 5'-OH), 5.13 (1H, t, H-2"a), 5.39 (1H, t, H-2"b), 5.70 (1H, m, H-3'), 5.74 (1H, d, *J* = 7.3 Hz, H-5), 6.60 (1H, d, *J* = 1.5 Hz, H-1'), 7.25 (1H, br s, NH), 7.28 (1H, br s, NH), 7.56 (1H, d, *J* = 7.3 Hz, H-6). *Anal.* Calcd for $C_{28}H_{47}N_3O_5$: C, 66.50; H, 9.37; N, 8.31. Found: C, 66.23; H, 9.20; N, 7.99.

***N*^{4}-Benzoyl-5'-*O*-*tert*-butyldimethylsilyl-2'-deoxy-2'-methylidenecytidine (9).** TBDMSCl (2.9 g, 19 mmol) was added to a solution of compound **3i** (6.0 g, 17 mmol) and imidazole (1.3 g, 19 mmol) in DMF (30 ml) at room temperature and the solution was stirred for 1 h. This solution was made basic with saturated sodium hydrogen carbonate solution, and extracted with CHCl$_3$. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a column of silica gel and eluted with CHCl$_3$-MeOH (50:1, v/v) to give **9** (7.2 g, 90%, crystallized from AcOEt); mp 123-128 °C. MS *m/z* (FAB): 458 [(M+H)$^+$]. ^{1}H NMR (CDCl$_3$) δ 0.13 (3H, s, Si-Me), 0.14 (3H, s, Si-Me), 0.95 (9H, s, Si-*t*-Bu), 2.80 (1H, br s, 3'-OH), 3.90-4.07 (3H, m, H-4',5'a,b), 4.80 (1H, d, H-3'), 5.51 (1H, t, H-2"a), 5.67 (1H, t, H-2"b), 6.85 (1H, d, *J* = 1.5 Hz, H-1'), 7.49-8.21 (7H, m, H-5,6 and benzoyl-H), 8.79 (1H, br s, NH). *Anal.* Calcd for $C_{23}H_{31}N_3O_5Si$: C, 60.37; H, 6.83; N, 9.18. Found: C, 60.07; H, 6.85; N, 8.93.

***N*^{4}-Benzoyl-3'-*O*-benzyl-5'-*O*-*tert*-butyldimethylsilyl-2'-deoxy-2'-methylidenecytidine (10).** NaH (60% in mineral oil, 0.55 g, 2.4 equiv.) was added to a solution of **9** (2.6 g, 5.7 mmol) in THF (15 ml) at room temperature and the mixture was stirred for 30 min. Benzyl bromide (0.81 ml, 6.8 mmol) was added and the reaction mixture was stirred at room temperature for 5 h. *p*-TsOH (1.5 g, 8.0 mmol) was added for neutralization, and water was added in an ice bath. This mixture was quickly extracted with CHCl$_3$. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (100:1, v/v) to give **10** (2.4 g, 77%, crystallized from ether-hexane); mp 125-127 °C. MS *m/z*: 547 (M^+). ^1H NMR ($CDCl_3$) δ 0.08 (3H, s, Si-Me), 0.10 (3H, s, Si-Me), 0.91 (9H, s, Si-*t*-Bu), 3.81-3.99 (2H, m, H-5'a,b), 4.18 (1H, dd, H-4'), 4.54-4.58 (2H, m, benzylic-H), 4.73 (1H, d, H-3'), 5.44 (1H, t, H-2"a), 5.47 (1H, t, H-2"b), 6.89 (1H, d, H-1'), 7.30-8.21 (12H, m, H-5,6, benzoyl-H and phenyl-H), 8.73 (1H, br s, NH). *Anal.* Calcd for $C_{30}H_{37}N_3O_5Si$: C, 65.79; H, 6.81; N, 7.67. Found: C, 65.58; H, 6.85; N, 7.52.

3'-*O*-Benzyl-2'-deoxy-2'-methylidenecytidine (11). A solution of **10** (2.6 g, 4.7 mmol) in THF (50 ml) was treated with TBAF (1M THF solution, 6ml, 6 mmol) at room temperature for 2 h. To the reaction mixture, MeOH (40 ml) and 28% aqueous ammonia (40 ml) was added, and the solution was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (10:1, v/v) to give **11** (1.0 g, 64%, crystallized from EtOH-H_2O); mp 70-72 °C. MS *m/z*: 329 (M^+). UV λ_{max} (H_2O) 269 nm (ε 7,900); UV λ_{min} (H_2O) 250 nm (ε 6,500); UV λ_{max} (0.05 N HCl) 277 nm (ε 11,600); UV λ_{min} (0.05 N HCl) 241 nm (ε 1,500); UV λ_{max} (0.05 N NaOH) 269 nm (ε 7,800); UV λ_{min} (0.05 N NaOH) 251 nm (ε 6,500). ^1H NMR (DMSO-d_6) δ 3.58 (2H, m, H-5'a,b), 3.96 (1H, dd, H-4'), 4.48 (1H, dd, H-3'), 4.56-4.65 (2H, m, benzylic-H), 5.03 (1H, br s, 5'-OH), 5.08 (1H, t, H-2"a), 5.47 (1H, t, H-2"b), 5.74 (1H, d, J = 7.3 Hz, H-5), 6.60 (1H, d, J = 1.5 Hz, H-1'), 7.22 (1H, br s, NH), 7.26 (1H, br s, NH), 7.29-7.37 (5H, m, phenyl-H), 7.52 (1H, d, J = 7.3 Hz, H-6). *Anal.* Calcd for $C_{17}H_{19}N_3O_4{\cdot}H_2O$: C, 58.78; H, 6.09; N, 12.10. Found: C, 58.96; H, 5.92; N, 11.93.

N^4-Benzoyl-5'-*O*-*tert*-butyldimethylsilyl-3'-*O*-*p*-chlorobenzyl-2'-deoxy-2'-methylidenecytidine (12). In a manner similar to that described for **10**, *p*-chlorobenzylation of **9** (4.2 g, 9.2 mmol) in THF (15 ml) with NaH (60% in mineral oil, 0.88 g, 2.4 equiv.), *p*-chlorobenzyl bromide (2.3 g, 11 mmol), and *p*-TsOH (2.4 g, 13 mmol) gave **12** as an oil (4.2 g, 79%). MS *m/z*: 581, 583 (M^+). ^1H NMR ($CDCl_3$) δ 0.09, (3H, s, Si-Me), 0.11 (3H, s, Si-Me), 0.91 (9H, s, Si-*t*-Bu), 3.81-3.99 (2H, m, H-5'a,b), 4.17 (1H, m, H-4'), 4.51 (1H, d, J = 11.7 Hz, benzylic-H), 4.51 (1H, m, H-3'), 4.68 (1H, d, J = 11.7 Hz, benzylic-H), 5.43 (1H, d, H-2"a), 5.46 (1H, d, H-2"b), 6.88 (1H, d, J = 1.0 Hz, H-1'), 7.26-8.19 (11H, m, H-5,6, phenyl-H and benzyl-H), 8.85 (1H, br s, NH).

3'-*O*-*p*-Chlorobenzyl-2'-deoxy-2'-methylidenecytidine (13). In a manner similar to that described for **11**, the reaction of **12** (0.34 g, 0.58 mmol) gave **13** (0.17 g, 80%, crystallized from EtOH-H_2O); mp 97-102 °C. MS *m/z*: 362, 364 (M^+). UV λ_{max} (H_2O) 269 nm (ε 7,900); UV λ_{min} (H_2O) 250 nm (ε 6,500); UV λ_{max} (0.05 N

HCl) 277 nm (ε 11,600); UV λ_{min} (0.05 N HCl) 241 nm (ε 1,500); UV λ_{max} (0.05 N NaOH) 269 nm (ε 7,800); UV λ_{min} (0.05N NaOH) 251 nm (ε 6,500). ^{1}H NMR (DMSO-d_6) δ 3.66-3.86 (2H, m, H-5'a,b), 4.04 (1H, m, H-4'), 4.50 (1H, d, J = 11.7 Hz, benzylic-H), 4.53 (1H, m, H-3'), 4.60 (1H, d, J = 11.7 Hz, benzylic-H), 5.16 (1H, s, H-2"a), 5.42 (1H, s, H-2"b), 5.83 (1H, d, H-5), 6.59 (1H, d, H-1'), 6.80 (1H, br s, NH), 7.23-7.29 (4H, m, phenyl-H), 7.38 (1H, d, H-6), 7.52 (1H, br s, NH). *Anal.* Calcd for $C_{17}H_{18}N_3O_4Cl\cdot 1/2H_2O$: C, 54.77; H, 5.13; N, 11.27. Found: C, 54.62; H, 4.98; N, 11.16.

N^4-Benzoyl-5'-*O*-*tert*-butyldimethylsilyl-3'-*O*-*p*-methoxybenzyl-2'-deoxy-2'-methylidenecytidine (14). NaH (60% in mineral oil, 0.19 g, 2.4 equiv.) was added to a mixture of **9** (0.9 g, 2.0 mmol) and sodium iodide (0.15 mg, 0.5 equiv.) in THF (20 ml) and the reaction mixture was stirred at room temperature for 30 min. *p*-Methoxybenzyl chloride (0.37 g, 2.4 mmol) was added, and the reaction mixture was stirred at room temperature overnight. This reaction mixture was neutralized with *p*-TsOH (0.53 g, 2.8 mmol), and water was added in an ice bath. This mixture was quickly extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (200:1, v/v) to give **14** (0.67 g, 59%, crystallized from ether); mp 133-135 °C. MS *m/z* (FAB): 578 [$(M+H)^+$]. ^{1}H NMR ($CDCl_3$) δ 0.09 (3H, s, Si-Me), 0.10 (3H, s, Si-Me), 0.92 (9H, s, Si-*t*-Bu), 3.79-3.99 (2H, m, H-5'a,b), 3.81 (3H, s, OMe), 4.15 (1H, m, H-4'), 4.49 (1H, d, J = 11.2 Hz, benzylic-H), 4.53 (1H, m, H-3'), 4.65 (1H, d, J = 11.2 Hz, benzylic-H), 5.44 (1H, d, H-2"a), 5.45 (1H, d, H-2"b), 6.88-8.21 (12H, m, H-1',5,6, phenyl-H and benzoyl-H), 8.65 (1H, br s, NH). *Anal.* Calcd for $C_{31}H_{39}N_3O_6Si$: C, 64.45; H, 6.80; N, 7.27. Found: C, 64.66; H, 6.79; N, 7.33.

N^4-Benzoyl-3'-*O*-*p*-methoxybenzyl-2'-deoxy-2'-methylidenecytidine (15). A solution of compound **14** (1.5 g, 2.6 mmol) in THF (20 ml) was treated with TBAF (1 M THF solution, 2.6 ml, 2.6 mmol) at room temperature for 1 h. The THF was removed *in vacuo*, and the residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (100:1, v/v) to give **15** (0.99 g, 83%, crystallized from AcOEt); mp 130-132 °C. MS *m/z* (FAB): 464 [$(M+H)^+$]. ^{1}H NMR ($CDCl_3$) δ 2.82 (1H, br s, 3'-OH), 3.75-4.00 (2H, m, H-5'a,b), 3.97 (3H, s, OMe), 4.14 (1H, m, H-4'), 4.55 (1H, d, J = 11.2 Hz, benzylic-H), 4.68 (1H, d, J = 11.2 Hz, benzylic-H), 4.68 (1H, dd, H-3'), 5.38 (1H, t, H-2"a), 5.51 (1H, t, H-2"b), 6.69 (1H, d, H-1'), 5.88-7.98 (11H, m, H-5,6, phenyl-H and benzoyl-H), 8.80 (1H, br s, NH). *Anal.* Calcd for $C_{25}H_{25}N_3O_6$: C, 64.79; H, 5.44; N, 9.07. Found: C, 64.63; H,5.37; N, 9.11.

N^4-Benzoyl-5'-*O*-benzyl-3'-*O*-*p*-methoxybenzyl-2'-deoxy-2'-methylidenecytidine (16). NaH (60% in mineral oil, 0.08 g, 2.4 equiv.) was added to a solution of **15** (0.37 g, 0.8 mmol) in THF (20 ml) at room temperature and the mixture was stirred for 30 min. Benzyl bromide (0.16 g, 1.0 mmol) was added, and the reaction mixture was stirred at room temperature overnight. This solution was neutralized with *p*-TsOH (0.21 g, 1.1 mmol), and water was added in an ice bath. This mixture was quickly extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (200:1, v/v) to give **16** (0.40 g, 91%) as a foam. MS *m/z*: 553 (M^+). ^1H NMR ($CDCl_3$) δ 3.68-3.91 (2H, m, H-5'a,b), 3.81 (3H, s, OMe), 4.06 (1H, m, H-4'), 4.53 (1H, d, J = 11.2 Hz, benzylic-H), 4.66 (1H, d, J = 11.2 Hz, benzylic-H), 4.55 (1H, m, H-3'), 5.30 (1H, t, H-2"a), 5.52 (1H, t, H-2"b), 5.35-5.43 (2H, m, benzylic-H), 6.37-7.94 (18H, m, H-1',5,6, NH, phenyl-H and benzoyl-H). *Anal.* Calcd for $C_{32}H_{31}N_3O_6$: C, 69.43; H, 5.64; N, 7.59. Found: C, 69.35; H, 5.58; N, 7.53.

5'-*O*-Benzyl-2'-deoxy-2'-methylidenecytidine (17). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 0.74 g, 3.3 mmol) was added to a solution of **16** (0.90 g, 1.6 mmol) in CH_2Cl_2 (16.4 ml) and water (0.9 ml). The mixture was stirred at room temperature for 6 h. After neutralization with saturated sodium hydrogen carbonate solution, the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (25:1, v/v) to give N^4-benzoyl-5'-*O*-benzyl-2'-deoxy-2'-methylidenecytidine (0.36 g, 51%, crystallized from AcOEt); mp 180-184 °C. MS *m/z*: 433 (M^+). ^1H NMR ($CDCl_3$) δ 3.76-3.96 (3H, m, H-4',5'a,b), 4.70 (1H, m, H-3'), 4.90 (1H, d, 3'-OH), 5.35-5.43 (3H, m, benzylic-H, H-2"a), 5.56 (1H, t, H-2"b), 6.37-7.94 (14H, m, H-1',5,6, NH, phenyl-H and benzoyl-H). *Anal.* Calcd for $C_{24}H_{23}N_3O_5$: C, 66.50; H, 5.35; N, 9.69. Found: C, 66.20; H, 5.31; N, 9.59. To a solution of the above compound (0.36 g, 0.83 mmol) in MeOH (50 ml), 28% aqueous ammonia (20 ml) was added, and the solution was stirred at room temperature for 3 h. The reaction mixture was evaporated *in vacuo* to dryness. The residue was applied on a column of silica gel and eluted with $CHCl_3$-MeOH (10:1, v/v) to give **17** (0.24 g, 88%) as a foam. MS *m/z*: 329 (M^+). UV λ_{max} (H_2O) 269 nm (ε 6,900); UV λ_{min} (H_2O) 251 nm (ε 5,500); UV λ_{max} (0.05 N HCl) 278 nm (ε 10,300); UV λ_{min} (0.05 N HCl) 241 nm (ε 1,200); UV λ_{max} (0.05 N NaOH) 269 nm (ε 7,000); UV λ_{min} (0.05 N NaOH) 251 nm (ε 5,700). ^1H NMR (DMSO-d_6) δ 3.65-3.78 (3H, m, H-4',5'a,b), 4.48 (1H, m, H-3'), 4.54 (2H, s, benzylic-H), 5.16 (1H, t, H-2"a), 5.32 (1H, t, H-2"b), 5.62 (1H, d, J = 7.3 Hz, H-5), 5.70 (1H, d, 3'-OH), 6.54 (1H, d, J =

1.5 Hz, H-1'), 7.18 (1H, br s, NH), 7.22 (1H, br s, NH), 7.29-7.38 (5H, m, phenyl-H), 7.44 (1H, d, *J* = 7.3 Hz, H-6). *Anal.* Calcd for $C_{17}H_{19}N_3O_4 \cdot 1/2H_2O$: C, 60.34; H, 5.81; N, 12.42. Found: C, 60.46; H, 5.77; N, 12.27.

Biological Studies. In the antitumor tests, female F1 of C57BL/6NJcl X DBA/2NJcl (BD_2F_1) mice, purchased from Clea Japan (Kanagawa), were intraperitoneally (i.p.) inoculated with 10^6 of P388 leukemia cells, kindly supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo. The mice were treated i.p. or orally with 0.1 ml of a compound solution or suspension per 10 g body weight daily from 1 day after inoculation for 9 days. The increase in life span (ILS) was calculated by the following formula:

$$\text{ILS}(\%) = (T-C)/C \times 100$$

where T is the mean survival time of the treated group and C is the mean survival time of the control group. The mean survival time in the control group ranged from 10.3 to 11.8 days in this study. The dose which showed 30% ILS obtained graphically was referred to the minimum effective dose. To measure drug concentration, BD_2F_1 mice fasted overnight were i.p. or orally given 100 mg of DMDC or 120 mg of 3'-*O*-benzyl DMDC (**11**), equivalent to 100 mg of DMDC, per kg body weight. Blood samples were obtained from four mice of each group 1 and 3 h after administration. DMDC and **11** were determined by HPLC. The rate of hydrolysis of 5'-*O*-acyl DMDC (**2**) by porcine liver esterase was determined according to the method of Nagai et al.10) Briefly, the 5'-*O*-acyl derivatives at a concentration of 0.12 mM were incubated with 0.0076 units (for **2b-d**), 0.76 units (for **2a, e-g**) or 76 units (for **2h**, DMDC) of a partially purified porcine liver esterase (Sigma) per ml of at 37 °C for 60 min. The enzymatic reaction was stopped by rapid cooling in an ice bath. DMDC formed during the enzymatic reaction and unchanged forms were determined by HPLC. Hydrolysis rate of the esters (k: $\text{min}^{-1} \cdot \text{unit}^{-1}$) was calculated according to the method of Nagai et al.10)

Analysis. Degradation of the esters or the benzyl derivatives and regeneration of DMDC were monitored by HPLC (CCPM apparatus, TOSOH) equipped with a variable-wavelength ultraviolet (UV) detector (UV-8000, TOSOH). All compounds were detected at 269 nm. A reverse-phase Inertsil ODS-2 column (6.0 x 150 mm, 5 μm) was used with various mobile phases with a flow rate of 1.0 ml/min as follows: esters **2a-c** were detected with 75% CH_3CN-0.05 M triethylammonium acetate (TEAA) buffer (pH 7.0) as the mobile phase, esters **2d-h** were detected with 92% CH_3CN-0.05 M TEAA buffer, and DMDC was detected with 1% CH_3CN-0.05 M TEAA buffer in the enzymatic hydrolysis rate experiments. In blood concentration experiments, 3'-*O*-benzyl DMDC (**11**) was detected with 30% CH_3CN-0.05 M TEAA buffer and DMDC was detected with 0.05 M TEAA buffer. The detector signal proceeded and was recorded with a reporting integrator (Sic-12).

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