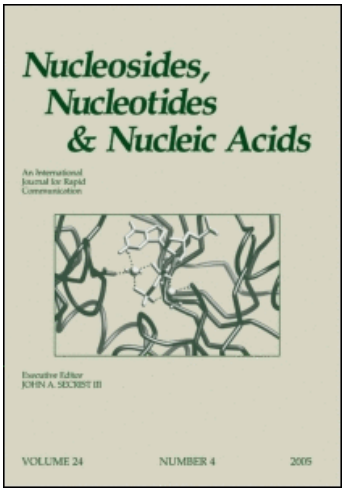

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Yamagat, Yuriko , Tomita, Ken-ichi , Marubayashi, Nobuhiro , Ueda, Ikuhiko , Sakata, Shinji , Matsuda, Akira , Takenuki, Kenji and Ueda, Tohru(1992) 'Molecular Conformation of 2'-Deoxy-2'-methylidene-cytidine: A Potent Antineoplastic Nucleoside', Nucleosides, Nucleotides and Nucleic Acids, 11: 2, 835 — 853
To link to this Article: DOI: 10.1080/07328319208021744
URL: http://dx.doi.org/10.1080/07328319208021744

NUCLEOSIDES AND NUCLEOTIDES, 11(2-4), 835-853 (1992)

MOLECULAR CONFORMATION OF 2'-DEOXY-2'-METHYLIDENE-CYTIDINE: A POTENT ANTINEOPLASTIC NUCLEOSIDE.

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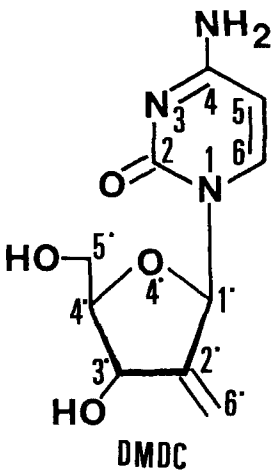
ABSTRACT: 2'-Deoxy-2'-methylidenecytidine (DMDC), a potent inhibitor of the growth of tumor cells, was crystallized with two different forms. One is dihydrated (DMDC·$2H_2O$) and the other is its hydrochloride salt (DMDC·HCl). Both crystal and molecular structures have been determined by the X-ray diffraction method. In both forms the glycosidic and sugar conformations are *anti* and C(4')-*exo*, respectively, whereas the conformation about the exocyclic bond is *trans* for DMDC·$2H_2O$ and *gauche*$^+$ for DMDC·HCl. Proton nuclear magnetic resonance data of DMDC indicate a preference for the *anti* C(4')-*exo* conformation found in the solid state. These molecular conformations were compared with the related pyrimidine nucleosides. When the cytosine bases are brought into coincidence, DMDC displays the exocyclic C(4')-C(5') bond located on the very close position to those of pyrimidine nucleosides with typical overall conformations. On the other hand, the hydroxyl O(3')-H groups are separated by ca. 3 Å in the cases of DMDC and other pyrimidine nucleosides which have the C(2')-*endo* sugar conformation. This result may be useful for the implication about the mechanism of the biological activity of DMDC.

INTRODUCTION

2'-Deoxy-2'-methylidenecytidine (DMDC), which constitutes an allylic alcohol system together with the 3'-secondary alcohol in the sugar moiety, has been found

This paper is dedicated to the memory of the late Professor Tohru Ueda.

to be highly active against not only leukemia and lymphoma cell lines but also adenocarcinoma and carcinoma cell lines in culture.[1,2] The broad spectrum of activity is novel among the nucleosides with antitumor activity. In addition to the antineoplastic activity *in vitro*, it has been reported that DMDC has therapeutic activity against human tumor xenografts.[3] As is known well, 1-(β-D-arabinofuranosyl)cytosine (ara-C) is one of the most potent drugs available for the treatment of adult acute myeloblastic leukemia.[4] The antileukemic mechanism of ara-C is the inhibition of DNA synthesis due to interference with DNA polymerase by the triphosphate derivative of ara-C. Major drawbacks in the clinical use of ara-C are its short lifetime in plasma due, in part, to deamination to inactive 1-(β-D-arabinofuranosyl)uracil (ara-U) by cytidine deaminase[5-7] and its ineffectiveness against solid tumors. In contrast to ara-C, DMDC is resistant to the cytidine deaminase from mouse kidney[1] and has good activity toward both leukemic and solid tumors as mentioned above. The mechanism of the antitumor activity of DMDC is considered the inhibition of DNA synthesis based on the inhibition of DNA polymerase by the triphosphate derivative[8] as well as in the case of ara-C[9] and/or the inhibition of ribonucleotide reductase by the diphosphate derivative.[10] The mechanism requires that DMDC is converted into the 5'-polyphosphates by nucleoside and nucleotide kinases. For the interaction of DMDC with enzymes such as deaminase and kinase, therefore, the overall molecular conformation as well as the overall shape of the nucleoside seems to be crucial. We determined the molecular conformations of two forms, DMDC·2H_2O and DMDC·HCl by X-ray diffraction and estimated the solution conformation of DMDC from proton nuclear magnetic resonance (^{1}H-NMR) spectra. These conformations were compared with those of the related pyrimidine nucleosides, especially pyrimidine arabinonucleosides. Furthermore, the molecular recognition mechanisms by target enzymes for the action of DMDC were discussed.



DMDC

EXPERIMENTAL

DMDC (DMDC·HCl) was synthesized as described in the previous paper[2] and crystallized from aqueous solution for DMDC·$2H_2O$ and from methanol–acetone solution for DMDC·HCl. The crystal data are as follows: DMDC·$2H_2O$, chemical formula $C_{10}H_{13}N_3O_4.2H_2O$, space group $P2_1$, a = 8.430(2), b = 15.335(3), c = 5.064(1) Å, β = 93.23(2)°, V = 653.6 Å^3, Z = 2 , D_x = 1.398 Mgm^{-3}, $F(000)$ = 292, μ(Cu $K\alpha$) = 0.951 mm^{-1}; DMDC·HCl, chemical formula $C_{10}H_{14}N_3O_4^+.Cl^-$, space group $P2_1$, a = 8.3109(7), b = 12.180(3), c = 6.0569(4) Å, β = 98.111(6)°, V = 607.0 Å^3, Z = 2, D_x = 1.508 Mgm^{-3}, $F(000)$ = 288, μ(Cu $K\alpha$) = 2.95 mm^{-1}. Both intensity data were measured on ENRAF–NONIUS CAD 4F–11 with Cu $K\alpha$ radiation using the ω–2θ scan method. 1605 reflections for DMDC·$2H_2O$ and 1060 reflections for DMDC·HCl within $\sin\theta/\lambda$ = 0.58 Å^{-1} were collected, of which 1140 for DMDC·$2H_2O$ and 944 for DMDC·HCl with $I > 2.3\sigma(I)$ were considered as observed ones. The intensities were corrected for Lorentz and polarization but not for absorption. The structures were solved by direct methods using program *MULTAN* 11/82[11] and refined by the block–diagonal least–squares method with anisotropic temperature factors for nonhydrogen atoms. All hydrogen atoms of DMDC molecule except one attached to O(5') in DMDC·$2H_2O$ and all hydrogen atoms of DMDC·HCl except one attached to N(6) were located on difference Fourier maps and refined with isotropic temperature factors. The final R values were 0.042 for DMDC·$2H_2O$ and 0.045 for DMDC·HCl. Scattering factors were taken from "*International Tables for X–ray Crystallography*".[12] All numerical calculations for X–ray analysis were performed on a VAX–11/750 using *SDP*[13] and *UNICS* III[14] systems.

^{1}H–NMR spectra of DMDC in DMSO–d_6 were recorded at 500.162 MHz on a JEOL–GX 500 spectrometer at 25°. The sample was prepared by lyophilizing DMDC·$2H_2O$ dissolved in D_2O. Chemical shifts were measured by using HDO as an internal standard (2.500 ppm). The resonance peaks were assigned as previously described[2] and the chemical shifts and coupling constants were confirmed by computer simulation.[51]

The crystallographic data for related nucleosides and nucleotides were obtained from the Cambridge Crystallographic Database,[15] and geometrical calculations and ORTEP

TABLE 1. Final atomic parameters of DMDC·$2H_2O$

$B_{eq}=4/3\Sigma_i\Sigma_j\beta_{ij}\mathbf{a}_i\cdot\mathbf{a}_j$

atom	x	y	z	$B_{eq/iso}$(Å^2)
N(1)	0.8157(3)	0.3062(2)	1.0067(5)	2.42(5)
C(2)	0.7393(3)	0.2254(2)	1.0129(6)	2.52(6)
N(3)	0.6175(3)	0.2080(2)	0.8329(5)	2.46(5)
C(4)	0.5760(3)	0.2674(2)	0.6492(6)	2.37(6)
C(5)	0.6515(3)	0.3506(2)	0.6394(7)	2.86(7)
C(6)	0.7712(4)	0.3661(2)	0.8176(7)	2.85(7)
O(2)	0.7841(3)	0.1711(2)	1.1832(5)	3.57(5)
N(4)	0.4599(3)	0.2478(2)	0.4706(6)	3.16(6)
C(1')	0.9549(3)	0.3224(2)	1.1875(6)	2.74(7)
C(2')	1.1105(3)	0.3184(2)	1.0516(7)	2.80(7)
C(3')	1.1903(3)	0.4056(2)	1.0905(6)	2.44(6)
C(4')	1.1057(3)	0.4410(2)	1.3281(6)	2.64(7)
C(5')	1.0989(4)	0.5378(2)	1.3478(8)	3.67(8)
C(6')	1.1680(5)	0.2479(3)	0.9425(11)	5.54(13)
O(3')	1.3575(2)	0.3971(2)	1.1346(5)	3.41(5)
O(4')	0.9450(2)	0.4094(2)	1.2827(5)	3.06(5)
O(5')	1.0153(3)	0.5628(2)	1.5750(6)	4.55(7)
O(W1)	0.4768(3)	0.0449(2)	0.9721(6)	3.74(6)
O(W2)	0.3158(3)	0.0703(2)	0.4614(5)	3.87(6)
H(O3')	1.412(4)	0.446(3)	1.090(8)	4.0(8)
H(N4)	0.403(5)	0.192(3)	0.464(9)	5.4(10)
H(N4')	0.430(4)	0.288(3)	0.327(8)	4.2(9)
H(5)	0.607(4)	0.394(3)	0.505(9)	5.3(10)
H(6)	0.834(4)	0.424(3)	0.804(8)	4.2(9)
H(1')	0.950(4)	0.279(3)	1.323(7)	3.4(8)
H(3')	1.150(3)	0.446(2)	0.926(7)	2.6(7)
H(4')	1.156(4)	0.409(3)	1.486(7)	3.5(7)
H(5')	1.210(5)	0.555(4)	1.389(10)	6.4(12)
H(5")	1.024(4)	0.562(3)	1.164(9)	4.9(9)
H(6')	1.102(4)	0.184(3)	0.952(8)	3.9(8)
H(6")	1.280(5)	0.254(4)	0.845(12)	7.4(13)

TABLE 2. Final atomic parameters of DMDC·HCl

$$B_{eq}=4/3\Sigma_i\Sigma_j\beta_{ij}\mathbf{a}_i\bullet\mathbf{a}_j$$

atom	x	y	z	$B_{eq/iso}$(Å^2)
N(1)	0.7391(4)	0.7122(3)	0.3650(5)	2.71(7)
C(2)	0.6792(4)	0.7918(4)	0.4970(7)	2.85(8)
N(3)	0.5787(4)	0.7498(3)	0.6408(5)	2.86(7)
C(4)	0.5440(4)	0.6431(4)	0.6649(7)	2.85(8)
C(5)	0.6064(5)	0.5657(4)	0.5248(8)	3.27(10)
C(6)	0.7031(5)	0.6031(3)	0.3818(7)	2.91(9)
O(2)	0.7125(4)	0.8877(3)	0.4910(6)	4.04(8)
N(4)	0.4531(5)	0.6126(4)	0.8195(7)	4.07(10)
C(1')	0.8616(5)	0.7507(3)	0.2237(6)	2.75(8)
C(2')	1.0324(5)	0.7456(4)	0.3435(6)	2.89(9)
C(3')	1.1166(5)	0.6541(4)	0.2417(7)	3.27(10)
C(4')	1.0158(5)	0.6464(4)	0.0086(7)	3.08(9)
C(5')	1.0108(6)	0.5339(4)	−0.0994(8)	3.98(12)
C(6')	1.0973(5)	0.8126(5)	0.5017(7)	4.07(12)
O(3')	1.2841(4)	0.6715(3)	0.2365(6)	4.65(9)
O(4')	0.8545(3)	0.6770(3)	0.0406(4)	3.23(6)
O(5')	0.9606(4)	0.4513(3)	0.0377(6)	4.65(9)
Cl(1)	0.4166(1)	0.8742(1)	1.0065(2)	3.94(2)
H(O3')	1.284(7)	0.741(7)	0.174(11)	7.7(18)
H(O5')	0.844(6)	0.443(5)	0.010(8)	3.8(10)
H(N3)	0.526(7)	0.802(6)	0.709(11)	6.9(16)
H(N4)	0.438(7)	0.525(5)	0.851(9)	5.3(13)
H(5)	0.577(5)	0.486(5)	0.518(8)	3.5(10)
H(6)	0.758(7)	0.549(6)	0.272(9)	5.3(7)
H(1')	0.831(4)	0.830(3)	0.180(6)	1.7(7)
H(3')	1.099(5)	0.582(4)	0.322(7)	3.1(9)
H(4')	1.060(5)	0.711(4)	−0.088(7)	2.8(8)
H(5')	0.936(7)	0.524(5)	−0.230(10)	6.2(14)
H(5")	1.129(6)	0.508(4)	−0.112(8)	3.4(9)
H(6')	1.039(7)	0.885(6)	0.553(10)	5.9(13)
H(6")	1.217(4)	0.796(4)	0.564(7)	2.5(8)

TABLE 3. Bond lengths (Å) and angles (°) with standard values

	DMDC·2H_2O	DMDC·HCl	C[a]/S-type[b]	C$^+$[a]/N-type[b]
N(1)–C(2)	1.398(4)	1.392(6)	1.399	1.381
C(2)–N(3)	1.360(4)	1.387(5)	1.356	1.387
N(3)–C(4)	1.334(4)	1.344(6)	1.334	1.352
C(4)–C(5)	1.429(4)	1.415(6)	1.426	1.413
C(5)–C(6)	1.337(4)	1.341(7)	1.337	1.341
C(6)–N(1)	1.364(4)	1.369(5)	1.364	1.362
C(2)–O(2)	1.243(4)	1.202(5)	1.237	1.211
C(4)–N(4)	1.329(4)	1.336(6)	1.337	1.313
N(1)–C(1')	1.468(4)	1.494(5)	1.463	1.484
C(1')–C(2')	1.517(4)	1.501(5)	1.526	1.530
C(2')–C(3')	1.504(5)	1.494(6)	1.528	1.525
C(3')–C(4')	1.532(4)	1.539(6)	1.526	1.519
C(4')–O(4')	1.445(4)	1.431(5)	1.453	1.449
C(1')–O(4')	1.424(4)	1.422(5)	1.415	1.409
C(4')–C(5')	1.489(5)	1.516(7)	1.514	1.507
C(2')–C(6')[O(2')]	1.320(6)	1.315(6)	1.409	1.419
C(3')–O(3')	1.421(3)	1.413(5)	1.424	1.414
C(5')–O(5')	1.435(5)	1.405(7)	1.423	1.420
C(2)–N(1)–C(6)	120.2(3)	122.2(4)	120.6	121.5
N(1)–C(2)–N(3)	119.3(3)	113.6(4)	118.9	114.9
C(2)–N(3)–C(4)	119.5(3)	125.6(4)	120.0	125.1
N(3)–C(4)–C(5)	122.3(3)	118.5(4)	121.8	117.5
C(4)–C(5)–C(6)	117.1(3)	117.7(4)	117.6	118.5
C(5)–C(6)–N(1)	121.5(3)	122.4(4)	121.0	122.5
N(1)–C(2)–O(2)	119.2(3)	123.8(4)	119.2	123.5
N(3)–C(2)–O(2)	121.5(3)	122.6(4)	121.9	121.6
N(3)–C(4)–N(4)	118.3(3)	119.7(4)	117.9	119.5
C(5)–C(4)–N(4)	119.4(3)	121.8(4)	120.3	123.3
C(2)–N(1)–C(1')	119.3(3)	116.0(3)		
C(6)–N(1)–C(1')	120.2(2)	121.4(3)		
N(1)–C(1')–C(2')	113.1(3)	112.6(3)	114.6	112.4
N(1)–C(1')–O(4')	108.0(2)	107.0(3)	108.3	108.8
O(4')–C(1')–C(2')	105.0(3)	106.4(3)	105.6	107.3
C(1')–C(2')–C(3')	107.3(3)	107.3(3)	101.1	101.2
C(2')–C(3')–C(4')	101.1(2)	101.4(3)	102.5	102.2
C(3')–C(4')–O(4')	103.3(2)	105.0(3)	106.4	104.3
C(4')–O(4')–C(1')	107.2(2)	109.5(3)	109.1	110.0
C(1')–C(2')–C(6')[O(2')]	124.7(3)	126.1(4)	112.7	107.7
C(3')–C(2')–C(6')[O(2')]	127.7(3)	126.5(4)	114.3	110.4
C(2')–C(3')–O(3')	111.8(3)	114.7(4)	114.1	110.0
C(4')–C(3')–O(3')	114.2(2)	113.5(4)	112.5	109.4
C(3')–C(4')–C(5')	115.3(3)	115.5(4)	115.7	114.9
C(5')–C(4')–O(4')	107.8(3)	109.0(4)	109.7	108.7
C(4')–C(5')–O(5')	110.1(3)	112.6(4)	111.1	111.8

[a]C and C$^+$ indicate mean values of the neutral and protonated cytosine residues, respectively.[17] [b]S-type and N-type indicate mean values of C(2')-*endo* and C(3')-*endo* riboses, respectively.

drawings[16] were carried out on an ACOS 930 computer at the Protein Engineering Research Center, Institute for Protein Research, Osaka University.

RESULTS AND DISCUSSION

The final atomic parameters of DMDC·$2H_2O$ and DMDC·HCl are listed in Tables 1 and 2, respectively.

(a) Bond lengths and angles

Bond lengths and angles are given in Table 3 with the standard values of the neutral and N(3) protonated cytosine residues[17] and the sugar moieties with S- and N-type puckering.[18] The bond lengths and angles of the cytosine moieties in DMDC·$2H_2O$ and DMDC·HCl are very similar to the standard values observed in the neutral and N(3) protonated cytosine residues, respectively (Table 3). In the two cytosine bases the most significant differences are found in the bond angles around N(3) and C(2). The C(2)–N(3)–C(4) bond angle of DMDC·HCl with the N(3) protonated cytosine moiety is larger by 6.1° than that of DMDC·$2H_2O$, whereas the N(1)–C(2)–N(3) bond angle of DMDC·HCl is smaller by 5.7°. The C(2)–O(2) and C(2)–N(3) bond lengths of DMDC·HCl also differ from those of DMDC·$2H_2O$. The delocalization of electrons in the fragment N(3)–C(2)–O(2) of DMDC·HCl is not as strong as that of DMDC·$2H_2O$.

The bond lengths and angles of both sugar moieties are in good agreement with each other except of the C(4')–C(5') and C(5')–O(5') bond lengths. As expected, the bond lengths and angles around C(2') taking an sp^2 hybridization in both 2'-deoxy-2'-methylideneribose moieties are different from those found in ribonucleosides.[18] The shortening of the C(1')–C(2') and C(2')–C(3') bonds compared to those of ribose is observed. The endocyclic C(1')–C(2')–C(3') bond angles are larger by ca. 6° than the standard one of ribose. The corresponding exocyclic bond angles around C(2') also increase by 12–17°.

(b) Molecular conformation

ORTEP[16] drawings of DMDC·$2H_2O$ and DMDC·HCl are illustrated in Fig. 1. Table 4 lists the selected torsion angles. The conformation about the glycosidic bond is, as usual, *anti* with the torsion angle [χ; C(2)–N(1)–C(1')–O(4')] of −140.1(3)° for DMDC·$2H_2O$ and −155.2(3)° for DMDC·HCl. In the pyrimidine ribo- and deoxyribonucleosides series, the *anti* conformation greatly dominates, except

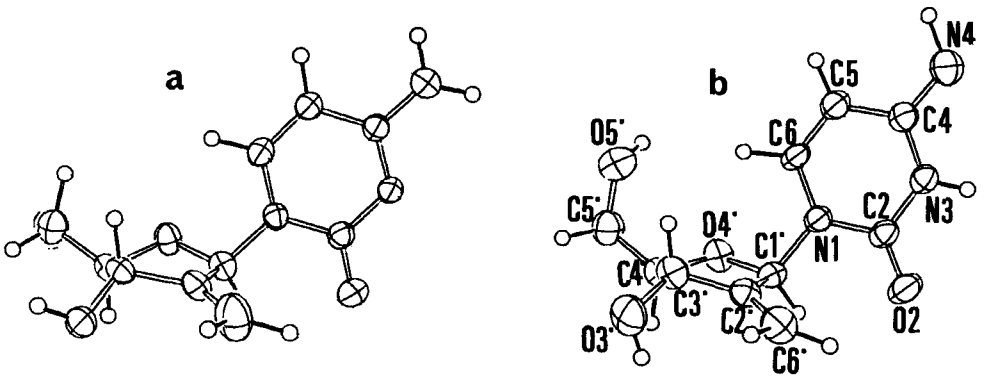


Fig. 1 *ORTEP* drawings of DMDC molecules and numbering system. (a); DMDC·2H_2O, (b); DMDC·HCl

TABLE 4. Selected torsion angles (°)

		DMDC·2H_2O	DMDC·HCl
χ	C(2)–N(1)–C(1')–O(4')	−140.2(3)	−155.2(3)
	C(6)–N(1)–C(1')–O(4')	46.2(4)	32.0(5)
τ_0	C(4')–O(4')–C(1')–C(2')	−27.5(3)	−13.3(4)
τ_1	O(4')–C(1')–C(2')–C(3')	3.3(3)	−8.1(4)
τ_2	C(1')–C(2')–C(3')–C(4')	12.0(3)	24.1(4)
τ_3	C(2')–C(3')–C(4')–O(4')	−36.1(3)	−31.9(4)
τ_4	C(3')–C(4')–O(4')–C(1')	40.7(3)	28.8(4)
ψ_{oc}	O(5')–C(5')–C(4')–C(3')	179.2(3)	53.6(5)
ψ_{oo}	O(5')–C(5')–C(4')–O(4')	64.5(3)	−64.2(5)

nucleosides substituted at C(6). The torsion angle χ is correlated with the sugar conformation.[19] The values of pyrimidine nucleosides and nucleotides with an N–type (C(3')–*endo*, P = −1° to 34°) sugar conformation are distributed in a range of −180° to −138° (*low anti* region), while the ones having an S–type (C(2')–*endo*, P = 137° to 194°) conformation display the value of −144° to −115° (*middle anti* region).[18] The ***high anti*** conformation (χ = −90° to −60°) found in purine nucleosides is not allowed because of the difference of steric hindrance at ***high anti*** region between H(6) of pyrimidine or

H(8) of purine and the sugar hydrogens. For pyrimidine arabinonucleosides, all of the glycosidic torsion angles are in the narrow range of −164° to −147°, although more than half of them take the S-type sugar conformation (see below), thereby avoiding the intramolecular short contacts between the H(6) atom and the 2'-"up" hydroxyl group of the arabinose moiety.

In both forms, the 2'-deoxy-2'-methylideneriboses have an unusual C(4')-*exo* conformation, of which the puckering of DMDC·HCl is distorted toward C(4')-*exo*, C(3')-*endo*. A similar sugar conformation was also found in the 2'-ketonucleoside, 4-ethoxy-1-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-β-D-*erythro*-2-pentofuranos-2-ulos-1-yl]-2(1*H*)-pyrimidinone,[20] which has an sp^2 hybridized C(2') atom like DMDC. The 2'-ketonucleoside crystallized in a *syn* form in spite of the C(6) unsubstituted pyrimidine nucleoside. The C(4')-*exo*, C(3')-*endo* pucker (P = 35° to 51°) is one of the most favorable sugar conformations for the *syn* form because of decreasing the steric hindrance between O(2) of pyrimidine bases and any atoms of sugar.[21] The pseudorotation parameters[22] of DMDC·$2H_2O$ and DMDC·HCl are P = 60.2° and τ_m = 40.3° and P = 41.6° and τ_m = 32.2°, respectively. These conformations are closer to the N-type puckering rather than the S-type and as expected in pyrimidine ribo- and deoxyribonucleosides, the values of χ are in the range of −180° to −138°. But the variation of the atomic distances between base atoms and sugar atoms when rotating the χ angle indicates that the χ angle must be allowed in the range of −180° to −120°. The conformation of the exocyclic C(4')-C(5') bond of DMDC·$2H_2O$ is *trans*, whereas DMDC·HCl adopts the *gauche*$^+$ conformation. In nucleosides the most preferred conformation is *gauche*$^+$, with the second being *trans*.

(c) Molecular packing and hydrogen bonds

The molecular packing and hydrogen bonds are shown in Fig. 2 for DMDC·$2H_2O$ and Fig. 3 for DMDC·HCl. In both crystals, all hydrogen atoms attached to the oxygen and nitrogen atoms participate in hydrogen bonds. The hydrogen bond parameters are listed in Table 5. In DMDC·HCl the Cl^- ion is hydrogen bonded to five kinds of donor groups, the amino, imino and hydroxyl groups of four surrounding DMDC molecules. In particular the hydrogen bond scheme involving cytosine bases and Cl^- ions is similar to that found in several other hydrochloride salts of cytosine derivatives, except that neighboring cytosine cations are related by a translation rather than by a two fold screw

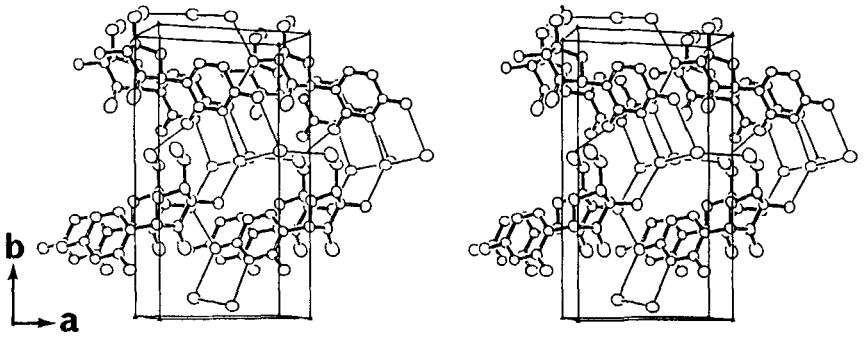


Fig. 2 Stereoview of the molecular arrangement in a unit-cell for DMDC·$2H_2O$. Thin lines indicate hydrogen bonds.

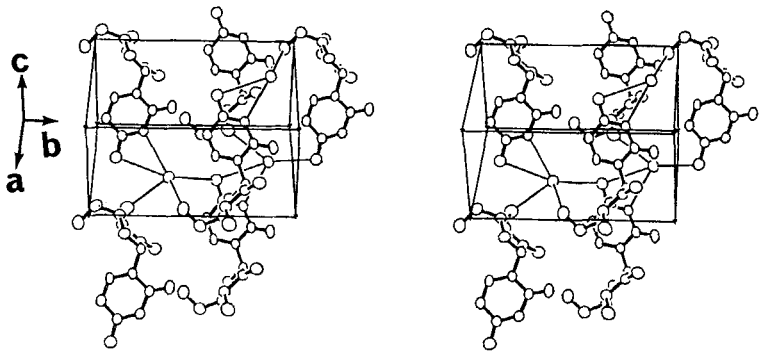


Fig. 3 Stereoview of the molecular arrangement in a unit-cell for DMDC·HCl. Thin lines indicate hydrogen bonds.

axis.[23-25] The arrangement with a continuous chain of N–H---Cl hydrogen bonds seems to be characteristic of hydrochloride salts of cytosine derivatives as pointed out by Sherfinski and Marsh.[23] In DMDC·$2H_2O$ two water molecules occupy the space between four DMDC molecules. The crystal structure displays an extensive network of hydrogen bonds containing eight kinds of hydrogen bonds between intermolecules of DMDC, between water molecules, and between water and DMDC molecules. There are no base stackings in either crystal.

(d) NMR analysis

^{1}H-NMR data are given in Table 6. The characteristic feature is the observation of long-range couplings between hydrogen atoms in the sugar. In general, the sugar

TABLE 5. Hydrogen bond paramerters

		A---D (Å)	H---D (Å)	A-H-D (°)
DMDC·2H_2O				
O(3')-H(O3')---O(W2)	2-x,1/2+y,2-z	2.733	1.82	176
N(4)-H(N4')---O(3')	-1+x,y,-1+z	2.953	2.01	161
N(4)-H(N4)---O(W1)	x,y,z	2.980	2.00	172
O(5')-H---O(2)[a]	2-x,1/2+y,3-z	2.623		
O(W1)-H---N(3)[a]	x,y,z	2.873		
O(W1)--H--O(W2)[a]	x,y,-1+z	2.918		
O(W2)--H--O(W1)[a]	x,y,z	2.879		
O(W2)-H---O(5')[a]	1-x,-1/2+y,2-z	2.789		
DMDC·HCl				
O(3')-H(O3')---Cl	-1+x,y,1+z	3.111	2.27	149
N(3)-H(N3)---Cl	x,y,z	3.140	2.31	154
N(4)-H---Cl[a]	x,y,z	3.409		
N(4)-H(N4)---Cl	1-x,1/2+y,2-z	3.224	2.30	141
O(5')-H(O5')---Cl	1-x,1/2+y,1-z	3.247	2.31	163

[a]Hydrogen positions were not located on the difference Fouriers.

TABLE 6. ^{1}H Chemical shifts (δ in ppm) and coupling constants (J in Hz) for DMDC

	δ		J
H(5)	5.714	H(5')-H(6')	7.5
H(6)	7.479	H(1')-H(3')	1.6[a]
H(1')	6.519	H(1')-H(6')	-1.8[b]
H(3')	4.445	H(1')-H(6")	-1.5[b]
H(4')	3.595	H(3')-H(4')	7.3
H(5')	3.694	H(3')-H(6')	-2.2[b]
H(5")	3.561	H(3')-H(6")	-2.6[b]
H(6')	5.305	H(4')-H(5')	2.2
H(6")	5.141	H(4')-H(5")	4.8
		H(5')-H(5")	-11.2
		H(6')-H(6")	<0.4

[a]A sign could not be determined. [b]Signs were estimated from the approch of Barfield et al.[30]

conformations in solution for ribo- and deoxyribonucleosides have been represented by an equilibrium between C(3')-*endo* (N) and C(2')-*endo* (S) types being typical two conformations found in crystal structures. The population of C(3')-*endo* (N) type can be evaluated by the method of Davies-Danyluk,[26] % of C(3')-*endo* = 100 $J_{3'4'}$ / ($J_{1'2'}$ + $J_{3'4'}$). The arabinonucleoside analogs have been treated similarly in equilibrium calculations,[27] or the favored sugar puckerings have been provided by the calculation of the corresponding dihedral angles from the vicinal coupling constants using Karplus relation.[28] DMDC with the 2'-methylidene group has only one vicinal coupling between H(3') and H(4') in the endocyclic sugar ring. The coupling constant $J_{3'4'}$ is 7.3 Hz and in comparable agreement with that (8.4, 8.6 Hz) calculated from the observed dihedral angle (θ = 147, 148° calculated from ideal hydrogen positions for DMDC·$2H_2O$ and DMDC·HCl, respectively) of H3'-C3'-C4'-H4' in the solid state using the modified Karplus equation.[29] The slight deviation between the observed and calculated coupling constants may reflect the contribution of another sugar pucker, but the ratio is small. In addition to the vicinal couplings, the conformational dependence for long-range H-H coupling constants over four bonds has been reported.[30] According to the approach by Barfield, the coupling constants over four bonds H-C(sp^2)-C(sp^2)-C(sp^3)-H depend on the dihedral angles (ϕ) between the planes formed by H-C(sp^2)-C(sp^2)-C(sp^3) and H-C(sp^3)-C(sp^2). The long-range coupling constants of H(1')-C(1')-C(2')-C(6')-H(6') [or H(6")] (-1.5 Hz or -1.8 Hz) and H(3')-C(3')-C(2')-C(6')-H(6') [or H(6")] (-2.2 Hz or -2.6 Hz) correspond to the ϕ values of ca. 50° and 80°, respectively, which agree well with those (46°, 54° and 82°, 80° calculated from ideal hydrogen positions) found in the crystal structures. This result also supports the preference for the C(4')-*exo* type pucker in solution. The conformation of the exocyclic bond is estimated as 70% *gauche*$^+$ and 30% others from the $J_{4'5'}$ and $J_{4'5''}$ values by the usual method.[26]

The conformation around the glycosidic bond can be estimated by using the intramolecular nuclear Overhauser effect (NOE) data. In DMDC, the NOE enhancements of H(5), H(1') and H(3') resonances upon saturating the H(6) resonance are 9%, 5% and 4%, respectively, while the corresponding enhancements of H(5) and H(1') in cytidine are 9% and 2% (unpublished result). The NOE value between H(5) and H(6) in DMDC is the same as that in cytidine but the one between H(6) and H(1') is more intense. This means that the weighted average distance between H(6) and H(1') in DMDC is somewhat

shorter than that of cytidine, *i.e.*, the ratio of the *syn* conformation which produces the shortening of the interatomic distance between H(1') and H(6) is somewhat larger than that in the case of cytidine. Assuming that the ratio of the NOE enhancements is almost equal to the inverse ratio of the involved internuclear distances to the sixth power, the ratio of weighted average distances between H(6) and H(1') and between H(6) and H(3'), 0.96 : 1.0 calculated from 5% and 4% NOEs, is consistent with the 20% population of *syn* (80% *anti*) with the C(4')-*exo* puckering. In this calculation the distances of 2.2 Å [H(1')-H(6), *syn*], 5.6 Å [H(3')-H(6), *syn*], 3.7 Å [H(1')-H(6), *anti*] and 2.9 Å [H(3')-H(6), *anti*] were used.

(e) Biological implications

We have found that the 5'-triphosphate of DMDC inhibits DNA polymerase,[8] and Baker et al. showed that the 5'-diphosphate derivative is an inactivator of the ribonucleoside diphosphate reductase.[10] These actions can explain that DMDC appears to inhibit cellular DNA synthesis of L1210 leukemia cells. According to the mechanisms, DMDC must be phosphorylated by deoxycytidine kinase in the first step in order to exhibit the potent antitumor activity.

Ara-C is known to be converted into 1-β-D-arabinofuranosylcytosine 5'-monophosphate (ara-CMP) by deoxycytidine kinase. Recently an extensive study of the substrate specificity of human deoxycytidine kinase performed by Eriksson et al. indicates that an amino group in the pyrimidine base appears to increase the efficiency significantly and the cytosine nucleoside analogs with very different sugar moieties, *e.g.*, cytidine, deoxycytidine, 2',3'-dideoxycytidine, 2'-deoxy-2'-fluorocytidine and acyclic compounds like cytallene can be phosphorylated.[31] This result suggests that human deoxycytidine kinase may recognize the amino group of the cytosine base and the exocyclic C(5')H_2OH group but not other sugar parts. It is reasonable to elucidate that the proposed recognition sites are located in relatively similar positions in the cases of deoxycytidine, ara-C and DMDC. Human deoxycytidine kinase can also phosphorylate purine deoxyribonucleosides, but the modifications of the deoxyribose lead to drastically decreased activity.[31] The difference of several kinetic and thermal properties between deoxycytidine kinase activity and deoxyadenosine kinase activity was found, and the existence of different conformational states for both activities has been suggested.[32] In the present studies we focused on the deoxycytidine kinase activity.

TABLE 7. Comparison of conformational paramaters in pyrimidine arabinonucleosides

compound	χ(°)	*P*(°)	τ_m(°)	ψ(°)	hydrogen bond	ref.
2'F-ara-I^{5}C[a]	-160.9	10.3	38.4	58.7	no	36
ara-CMP	-152.3	12.6	38.7	53.3	no	37
ara-S^{4}U[b]	-147.6	13.8	39.0	62.3	no	38
ara-Pr^{5}U[c]	-153.7	28.3	31.7	41.4	no	39
5'Cl-araC[d]	-150.6	33.5	36.8	60.7	no	40
ara-T[e]	-156.4	104.8	41.0	60.2	no	41
ara-Br^{5}U[f]	-150	108		61	no	42
5'CH_2OH-ara-C[g]	-161.2	145.6	39.6	173.6	no	23
ara-U	-148.4	153.9	38.6	55.8	yes	43
ara-NO_2^{5}U[h]	-155.3	158.2	34.9	51.6	yes	44
ara-C	-150.0	162.7	36.3	51.7	yes	45
ara-Me^{5}C[i]	-161.5	162.7	39.5	-163.8	no	46
5'N_3-ara-C[j]	-156.2	163.5	36.6	-69.6	no	47
ara-F^{5}C[k]	-164.1	165.0	37.3	173.8	no	48
3'OMe-ara-C[l]	-159.8	167.8	37.6	-166.6	no	49
ara-C.HCl	-156.1	176.9	34.3	-171.5	no	50

[a]2'-fluoro-5-iodo-arabinosylcytosine, [b]1-β-D-arabinofuranosyl-4-thiouracil, [c]5-(propyn-1-yl)-1-(β-D-arabinofuranosyl)uracil, [d]5'-chloro-5'-deoxyarabinofuranosylcytosine, [e]1-β-D-arabinofuranosylthymine, [f]1-β-D-arabinofuranosyl-5-bromouracil, [g]5'-hydroxymethyl-5'-deoxy-1-β-D-arabinofuranosyl-3*H*-cytosine, [h]5-nitro-1-β-D-arabinofuranosyluracil, [i]1-β-D-5-methylarabinofuranosylcytosine, [k]5-fluoro-arabinosylcytosine, [j]5'-azido-5'-deoxy-1-β-D-arabinofuranosylcytosine, [l]3'-O-methyl-1-β-D-arabinofuranosylcytsine.

On the other hand, DMDC is resistant to mouse kidney cytidine deaminase by which deoxycytidine, ara-C and 2'-fluoro-2'-deoxy-1-β-D-arabinofuranosylcytosine are deaminated. Cytosine, cytidine monophosphates, 3'-amino-2',3'-dideoxycytidine[33] and several analogs of ara-C having the 2'-"up" substituents such as azide, amino and *O*-nitro are not deaminated.[34,35] It is, therefore, expected that the enzyme exactly recognizes the difference of shape and conformation between substrates and nonsubstrates.

We summarized the conformations of pyrimidine arabinonucleoside and nucleotide analogs for consideration of the permissible models for ara-C in Table 7.[24,36-50] As mentioned above, the observed χ range is remarkably narrow (ca. 20°), and there is no correlation between the χ value and the sugar pucker. Their sugar puckers are

classified into three types, C(2')-*endo* (9 cases), C(3')-*endo* (5 cases) and unusual O(4')-*endo*, C(1')-*exo* (2 cases) puckerings. The unusual O(4')-*endo*, C(1')-*exo* pucker is also observed in a few deoxyribonucleosides, but in no ribonucleosides.[18] It is due to no eclipsing hydroxyl groups in deoxyriboses as well as in arabinoses. The exocyclic C(4')-C(5') conformation adopts *gauche*$^+$ or *trans* in the case of C(2')-*endo* type. On the other hand, the overall conformation of DMDC indicates from the X-ray structures and the NMR analysis that the preferable sugar puckering is C(4')-*exo* and the glycosidic conformation is more flexible than that of cytidine.

In order to investigate the conformational similarity and discrepancy between DMDC and the related nucleosides (ara-C, deoxycytidine, cytidine), we considered three kinds of overall molecular conformations in ara-C and two kinds of conformations in deoxycytidine[52] and cytidine[53,54] and superimposed DMDC·$2H_2O$ or DMDC·HCl on the seven kinds of conformations as shown Fig. 4. Surprisingly, when the bases are brought into coincidence, the exocyclic CH_2OH groups are very close to each other in all cases, except in the case of the model with *middle anti* and O(4')-*endo* conformations. It is unknown at present which overall conformation is favorable on the recognition by deoxycytidine kinase. However it seems that Fig. 4 displays that DMDC would be phosphorylated by the enzyme as well as deoxycytidine, cytidine and ara-C, because the cytosine base and the exocyclic CH_2OH group must be involved in the recognition sites by the enzyme but other parts of sugar may not be regarded. As shown by Eriksson et al.,[31] 2'-deoxy-2'-fluorocytidine which has the high preference for the C(3')-*endo* pucker[55] is the best substrate in the pyrimidine nucleosides they surveyed. That observation suggests that the model with the *low anti* and C(3')-*endo* type conformations may be preferred for the recognition and/or the phosphorylation by the enzyme, so that DMDC could be phosphorylated easily. Furthermore, the phosphorylated DMDC would be converted into the active forms, 5'-polyphosphate derivatives, by nucleotide kinases.

Although preliminary crystallographic data for *Escherichia coli* cytidine deaminase has been reported,[56] no three-dimensional structures of cytidine deaminase have been reported. However, the substrate specificities of several cytidine deaminases suggest that the sugar moiety plays an important role for the recognition by the enzymes as well as

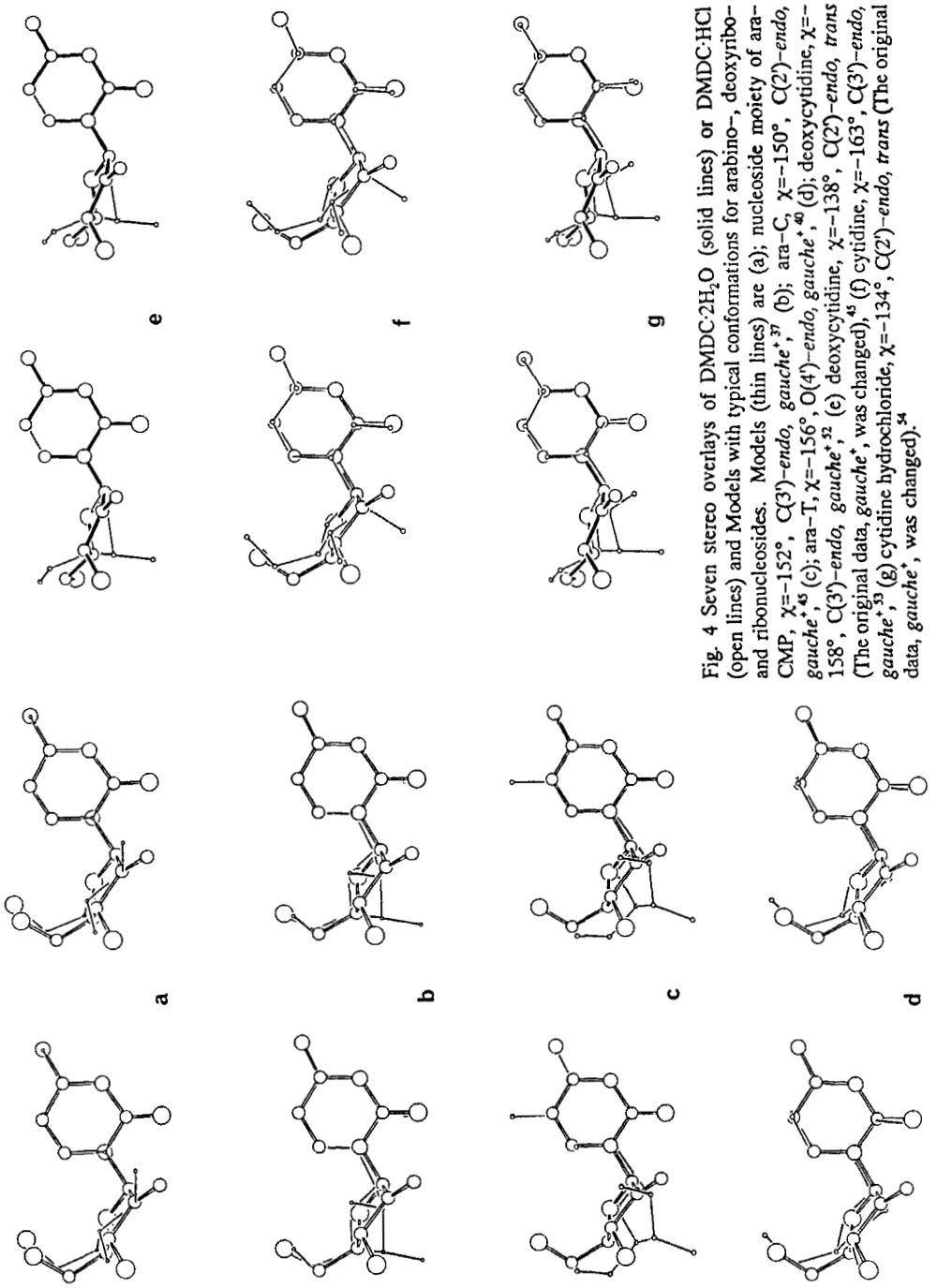


Fig. 4 Seven stereo overlays of DMDC·$2H_2O$ (solid lines) or DMDC·HCl (open lines) and Models with typical conformations for arabino-, deoxyribo- and ribonucleosides. Models (thin lines) are (a); nucleoside moiety of ara-CMP, χ=−152°, C(3')-*endo*, *gauche*$^+$;[37] (b); ara-C, χ=−150°, C(2')-*endo*, *gauche*$^+$;[45] (c); ara-T, χ=−156°, O(4')-*endo*, *gauche*$^+$;[40] (d); deoxycytidine, χ=−158°, C(3')-*endo*, *gauche*$^+$;[52] (e) deoxycytidine, χ=−138°, C(2')-*endo*, *trans* (The original data, *gauche*$^+$, was changed),[45] (f) cytidine, χ=−163°, C(3')-*endo*, *gauche*$^+$;[53] (g) cytidine hydrochloride, χ=−134°, C(2')-*endo*, *trans* (The original data, *gauche*$^+$, was changed).[54]

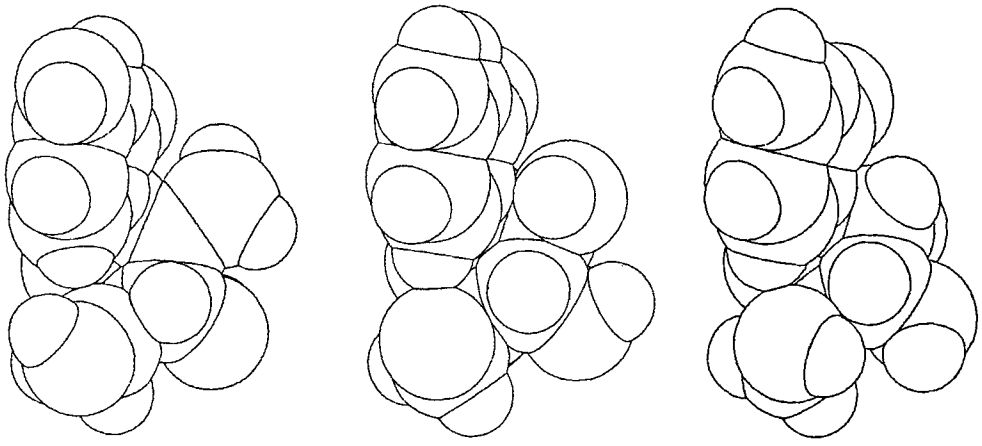


Fig. 5 Space filling models of DMDC·HCl, ara-C and cytidine.

the cytosine moiety. It is also assumed from the recent three-dimensional structure of adenosine deaminase complexed with 6*R*-hydroxyl-1,6-dihydropurine ribonucleoside, a transition state analog, which shows that there are extensive hydrogen bonds formed between the enzyme and the ribose moiety.[57] Assuming that the preferred conformation for the recognition is *middle anti* and C(2')-*endo* about the glycosidic bond and the sugar puckering, respectively, the position of the O(3')-H group which is one of candidates for the recognition site is significantly different between DMDC and other substrates. The separation distances are ca. 2.6-2.9 Å. The separation could not be complemented even if the glycosidic bond of DMDC is rotated in the some range. If the *low anti*, C(3')-*endo* form is recognized by the enzyme, the 2'-methylidene group of DMDC may result in steric hindrance on the interaction with the enzyme. As shown in Fig. 5, space filling models[58] of DMDC and *low anti*, C(3')-*endo* models of ara-C and cytidine reveal that the 2'-methylidene group of DMDC pushes out to the area of the enzyme compared with the 2'-hydroxyl groups of ara-C and cytidine and may interfere with the recognition of DMDC by the enzyme. Thus, DMDC may be resistant to cytidine deaminase.

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Received 10/25/91
Accepted 11/20/91