Nucleosides and Nucleotides. 97. Synthesis of New Broad Spectrum Antineoplastic Nucleosides, 2'-Deoxy-2'-methylidenecytidine (DMDC) and Its Derivatives[1]

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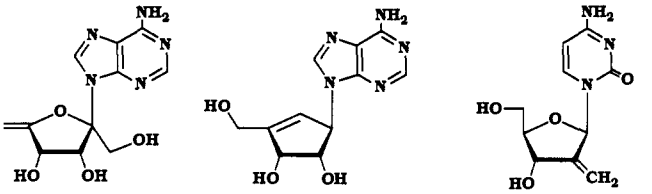
A new type of antineoplastic nucleoside, 2'-deoxy-2'-methylidenecytidine (DMDC) has been synthesized from the corresponding 2'-keto pyrimidine nucleosides **3** and **8** by the Wittig reaction. During the course of the reaction, we found that an intermediate betaine could pick a proton from the excess triphenylphosphonium bromide to form the 2'-phosphonium salts **5** and **10**, which could be further converted into the 2'-deoxy-2'-methylidene nucleosides **4** and **9** by treatment with sodium hydride. Various 5-substituted DMDC derivatives **19a–e,h** and their uracil congeners **16a–h** were also synthesized from the corresponding 5-substituted uridines **12a–f,h**. Among them, DMDC as well as 2'-deoxy-2'-methylidene-5-fluorocytidine (**19a**) showed potent antileukemic activity against murine L1210 cells in culture. The activity of DMDC and **19a** toward various human tumor cells in culture compared with 1-β-D-arabinofuranosylcytosine and 5-fluorouracil was also examined. In vivo antitumor activity of DMDC against L1210 was also described.

Certain analogues of 2'-deoxycytidine bearing a 2'-"up"-substituent are much more effective in terms of biological activity than those having a 2'-"down"-substituent. Although 1-β-D-arabinofuranosylcytosine (ara-C) is a potent inhibitor of growth of the leukemic cells, it shows only weak activity toward solid tumor cells. A major drawback in clinical use of ara-C against acute myeloblastic leukemia[2-4] is a short half-life time in plasma due, in part, to the deamination to the chemotherapeutically inactive 1-β-D-arabinofuranosyluracil.[5-7] It has also been reported that 2'-deoxy-2'-fluoro-β-D-arabinofuranosylcytosine[8,9] is a potent cell-growth inhibitor of murine leukemic L5178Y cells, although it is susceptible to the deaminase.[10] We have reported that 2'-azido-2'-deoxy-β-D-arabinofuranosylcytosine (Cytarazid), which is resistant to deamination by human cytidine deaminase,[11-13] has a broad spectrum of antineoplastic activity against various human tumor cells in culture, including solid tumor cells.[14] However, (2'*S*)-1-(2-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)cytosine has a quite similar antineoplastic spectrum to that of ara-C.[15] Moreover 2'-deoxy-2'-(methylthio)-β-D-arabinofuranosylcytosine is a weak inhibitor of murine leukemic cells in culture.[16] Considering the nature of the substituents at the arabino position of 2'-deoxycytidine, both bulkiness and polarity seem to be significant in affecting biological activity. When such a nucleoside analogue acts as an antimetabolite, it must be phosphorylated to some extent at the 5'-hydroxyl group by corresponding nucleoside kinases. For such enzyme recognition, therefore, the overall shape of the nucleoside, including the sugar conformation, glycosyl torsion angle, and spatial position of the 5'-hydroxyl group, seems to be critical. Moreover, bulkiness and electronegativity of the 2'-substituents might affect the sugar conformation and chemical reactivity of the 3'-hydroxyl group. These considerations, together with the nature of the 3'-hydroxyl group that would mainly be affected by the 2'-substituent, should also be important when such a nucleoside 5'-triphosphate is to be incorporated into DNA molecules by action of DNA polymerases. From these considerations, one approach to design nucleoside analogues that exert a broad spectrum of activity toward both leukemic and solid tumors has been the construction of analogues that (a) are not substrates for cytidine deaminase, (b) are converted into the corresponding 5'-polyphosphates by nucleoside and nucleotide kinases, which inhibit ribonucleotide reductase and/or DNA polymerase and can also be incorporated into a DNA molecule, (c) have a chemically reactive functionality at the 2'-position of 2'-deoxycytidine, which is stable at the nucleoside stage but would be expected to be reactive after incorporation into a DNA molecule. As an example of such compounds that fulfill, or partly fulfill, these requirements, we designed 2'-

Chart I



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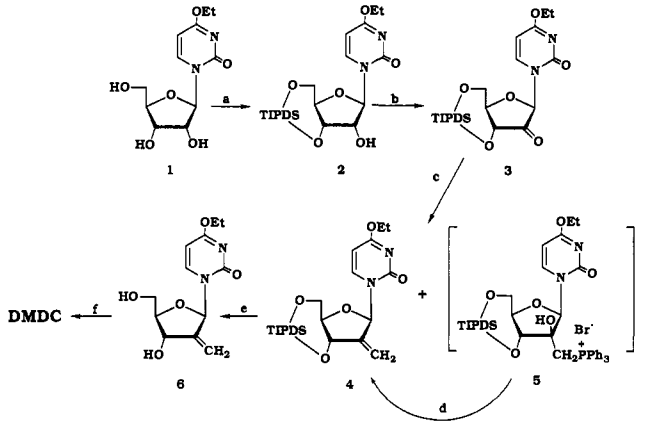
Scheme I[a]



[a] (a) $TIPDSCl_2$, pyridine; (b) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2; (c) $Ph_3P^+CH_3\ Br^-$, BuLi, THF; (d) NaH, THF; (e) TBAF, THF; (f) NH_3/MeOH then HCl.

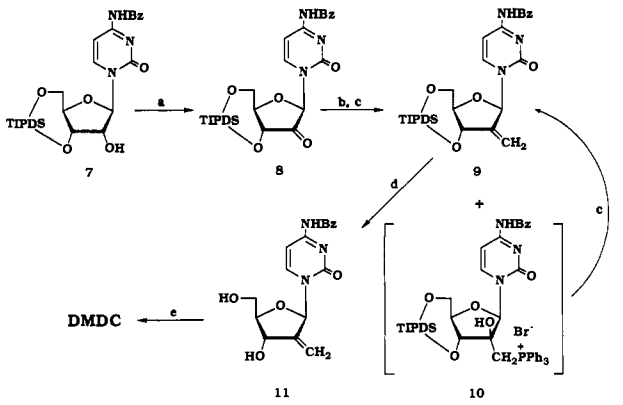
deoxy-2'-methylidenecytidine (DMDC),[17] which constitutes an allylic alcohol system together with the 3'-secondary alcohol group in the sugar moiety. When its nucleotide is incorporated into DNA molecules, the allylic alcohol system becomes a more reactive an allyl phosphate ester. The allylic alcohol system is found in nucleoside antibiotics such as angustmycin A[18,19] and neplanocin A[20] (Chart I), in which this structural feature may be important in the biological activity due to enhanced chemical reactivity and/or fixation of the sugar conformation.

We have reported the synthesis of 2'-deoxy-2'-methylidenecytidine (DMDC) from uridine, and DMDC was found to be active against not only mouse and human leukemic cell lines but also human adenocarcinoma and carcinoma cell lines in culture.[17] In this report, we give full accounts of the detailed synthesis of DMDC and its other pyrimidine congeners. We also report some structure–activity relationships of DMDC derivatives against the mouse leukemic cell line L1210. Moreover, we compare the antineoplastic activity of DMDC, 2'-deoxy-2'-methylidene-5-fluorocytidine (FDMDC), and its 5-methylcytidine derivative, MDMDC with ara-C and 5-fluorouracil (5-FU) toward various tumor cell lines including human adenocarcinoma and carcinoma cell lines in vitro.

Chemistry

Originally, we reported the synthesis of DMDC in eight steps from uridine as shown in Scheme I.[17] In this method, uridine has been transformed into 4-ethoxy-2-(1*H*)-pyrimidinone nucleoside **1**,[21,22] the 3',5'-hydroxyl group of which was then selectively protected by a tetraisopropyldisiloxane-1,3-diyl (TIPDS) group[23] to afford **2**.

Scheme II[a]



[a] (a) CrO_3/pyridine/Ac_2O, CH_2Cl_2; (b) $Ph_3P^+CH_3\ Br^-$, BuLi, THF; (c) NaH, THF; (d) TBAF, THF; (e) NH_3/MeOH.

Compound **2** was converted to the corresponding 2'-keto derivative **3** by Swern oxidation,[22,24] which was used for the Wittig reaction by using methylenetriphenylphosphorane (3 equiv prepared by the reaction of 4 equiv of methyltriphenylphosphonium bromide and 3 equiv of potassium hydride in dimethyl sulfoxide). From this reaction mixture, the desired 2'-deoxy-2'-methylidene nucleoside **4** was obtained in only 41% yield. Even if the reaction was done under forced conditions and a longer reaction time or if butyllithium was used instead as a base in tetrahydrofuran (THF), the yield of **4** could not be improved. During the course of the reaction by using careful checking with thin-layer chromatography (TLC), we however found an additional nucleosidic spot near the origin on the TLC plate after complete consumption of **3**. This polar nucleoside **5** was obtained in 51% yield after partial purification by silica gel column chromatography, and the ^1H NMR showed 15 phenyl protons as a multiplet at around 7.5–7.8 ppm. A peak at δ 23.44 ppm,[25] assigned as a phosphonium salt, was observed as a singlet in its ^{31}P NMR spectrum. Additionally, treatment of **5** with NaH in THF furnished the 2'-methylidene compound **4** in 76% yield (total yield of **4** was 78%). Therefore, the polar nucleoside was tentatively concluded to be the phosphonium salt **5**. From these observation, it seems that the oxaphosphetane formation required for the Wittig methylenation could be difficult because of the ring strain and the rather stable betaine could pick up a hydrogen from the excess of methyltriphenylphosphonium bromide to form the phosphonium salt **5**.

Treatment of **4** with tetrabutylammonium fluoride (TBAF) in THF afforded 4-ethoxy-1-(2-deoxy-2-methylidene-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**6**), which was further converted into 2'-deoxy-2'-methylidenecytidine (DMDC) by heating with methanolic ammonia in a sealed tube. The overall yield of DMDC from uridine was 32%. Since this method for the preparation of DMDC is rather lengthy, we devised an improved method starting from cytidine (Scheme II).

N^4-Benzoyl-1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]cytosine (**8**), which was prepared from N^4-benzoyl-1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]cytosine (**7**) by oxidation with the CrO_3/pyridine/Ac_2O complex (1:2:1 molar ratio)[26] in dichloromethane, was treated with methylene-

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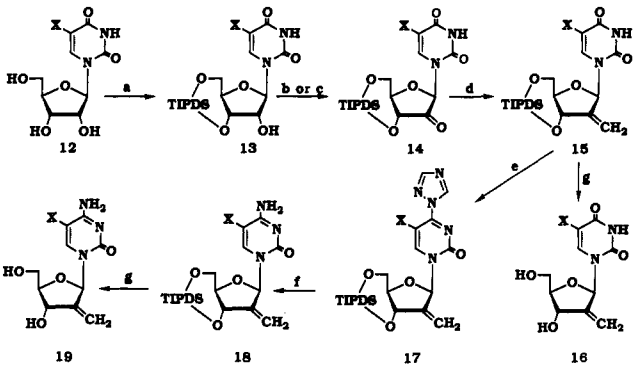
Scheme III[a]



a series X = F. b series X = Cl. c series X = Br. d series X = I.
e series X = Me. f series X = C ≡ CH. g series X = Et. h series X = H

[a] (a) $TIPDSCl_2$, pyridine; (b) CrO_3/pyridine/Ac_2O, CH_2Cl_2; (c) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2; (d) $Ph_3P^+CH_3$ Br^-, BuLi, THF; (e) 1,2,4-triazole, $POCl_3$, Et_3N, CH_3CN; (f) NH_3; (g) TBAF, THF.

triphenylphosphorane (prepared by reaction of 4 equiv of butyllithium and 4 equiv of methyltriphenylphosphonium bromide in THF). After complete consumption of the starting material as judged by TLC, the reaction mixture was acidified by the addition of aqueous ammonium bromide. To convert the phosphonium salt **10** into the desired 2′-methylidene derivative **9**, the crude reaction mixture was further treated with NaH in THF to afford **9** in 80% yield after purification with a silica gel column. Deprotection of **9** with TBAF with THF gave **11** as colorless crystals in 88% yield, which on treatment with methanolic ammonia furnished DMDC as a hydrochloride in 83% yield. Thus, DMDC was obtained at 36% overall yield in six steps from cytidine.

With DMDC as a lead compound, we extended our synthetic efforts to the preparation of several 5-substituted 2′-deoxy-2′-methylideneuridines and -cytidines with variation of the C-5 alkyl, ethynyl and halogeno substituents for studying structure–activity relationships (Scheme III). The 5-substituted uridines **12a–f,h** were likewise converted to the corresponding 3′,5′-*O*-TIPDS derivatives **13a–f,h**. The 5-ethyluracil derivative **13g** was obtained from the corresponding 5-ethynyl derivative **13f** by catalytic reduction. The 2′-hydroxyl group in **13a–d,f–h** was oxidized by the preformed complex of CrO_3/pyridine/Ac_2O (1:2:1) in CH_2Cl_2[26] to give the corresponding 2′-keto nucleosides **14a–d,f–h**. The conversion of **13e** to **14e** was effected by Swern oxidation. Reaction of **14a–h** with methylenetriphenylphosphorane afforded the 2′-deoxy-2′-methylidene nucleosides **15a–h**. After deprotection of these compounds with TBAF yielded the desired 2′-deoxy-2′-methylideneuridine derivatives **16a–h**. The corresponding cytosine nucleosides **19a–e,g** were obtained from **15a–e,g** via the 4-triazolo derivatives **17**[27] followed by treatment with NH_4OH. Removal of the TIPDS group with TBAF furnished 5-substituted 2′-deoxy-2′-methylidenecytosines **19a–e,g**.

Biological Activity

Among the series of 2′-deoxy-2′-methylideneuridines **16**, the 5-methyl derivative **16e** showed marginal activity against murine leukemia L1210 at a rather high concentration (see Table I). In contrast with 2′-deoxy-5-fluorouridine, 2′-deoxy-2′-methylidene-5-fluorouridine (**16a**) was completely inactive up to 100 μg/mL, probably due to inability of the latter to act as a substrate of nucleoside kinases. As 5-fluorouracil is an effective inhibitor of the growth of various tumor cells, this result may imply that **16a** could not be a substrate for nucleoside phosporylases. The ineffectivenss of 2′-deoxy-2′-methylidene-5-ethynyluridine (**16f**) toward L1210 cells might also be related to insusceptibility to nucleoside kinase, because 2′-deoxy-5-ethynyluridine 5′-monophosphate[28] is known to be a potent inhibitor of thymidylate synthetase. The other 5-halogenouracil derivatives, **16b**, **c**, and **d** were inactive at concentrations up to 100 μg/mL.

Table I. In Vitro Cytotoxicity of Pyrimidine 2′-Deoxy-2′-Methylidene Nucleosides against Murine L1210 Cells[a]

compds	IC_{50},[b] μg/mL
16h	>100
16a	>100
16b	>100
16c	>100
16d	>100
16e	50
16f	>100
16g	>100
DMDC	0.37
FDMDC	0.33
19b	>100
19c	>100
19d	>100
MDMDC	58.0
19g	>100

[a] Cytotoxic activity assay in vitro was done by the method of Carmichael et al.[32] L1210 cells (1×10^4/well) was incubated in the presence or absence of compounds for 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and the OD (570 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 − OD (570 nm) of sample well/OD (570 nm) of control well] 100. [b] IC_{50} μg/mL, was given as the concentration at 50% inhibition of cell growth.

On the other hand, the most notable among these in the cytosine series are DMDC and 2′-deoxy-2′-methylidene-5-fluorocytosine (**19a**, FDMDC), both of which are potent inhibitors of growth of L1210 cells at low concentrations. It is important to note from the above-mentioned results that FDMDC would act as a 5-fluorocytosine derivative but not as a 5-fluorouracil derivative via the deamination by cytidine deaminase. DMDC has been reported not to be a substrate of cytidine deaminase from mouse kidney.[17] The 5-methylcytosine derivative (**19e**, MDMDC) was about 170 times less active than DMDC as well as FDMDC; the IC_{50} value is comparable to its uracil congener. The other 5-halogenocytosines **19b–d** and the 5-ethylcytosine **19g** derivatives were found to be devoid of antileukemic activity against L1210 cells. It is conceivable that the order of antileukemic potency might be related to the substrate specificity of first activation enzymes such as thymidine kinase or deoxycytidine kinase.

We next examined the antineoplastic activity of DMDC, FDMDC, and MDMDC against various mouse and human lymphoma, leukemia, melanoma, adenocarcinoma, and carcinoma cell lines in vitro. The results are shown in Table II, which includes ara-C and 5-FU for comparison. From the data shown in Table II, it can be seen that ara-C is cytotoxic to various leukemia and lymphoma cells at rather lower concentrations, but not against human ade-

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Table II. Inhibitory Effects of DMDC, FDMDC, MDMDC, Ara-C, and 5-FU on the Growth of Various Mammalian Tumor Cell Lines in Vitro[a]

cell lines	IC_{50},[b] μg/mL				
	DMDC	FDMDC	MDMDC	Ara-C	5-FU
B16[c]	3.0	9.2	>100	0.24	2.6
3LL[d]	16.0	22.0	>100	ND[s]	ND[s]
CCRF CEM[e]	0.067	0.1	>1	0.32	40
MOLT-4[f]	0.041	0.030	>100	0.056	3.8
HL-60[g]	0.040	0.053	3.0	>0.1	1.3
U-937[h]	0.44	0.40	>10	0.46	3.5
PC-6[i]	39.0	16.0	>100	50.0	ND[s]
PC-10[j]	>100	>100	>100	>100	>100
PC-13[k]	1.0	1.4	>100	>100	ND[s]
PC-14[l]	>100	>100	>100	>100	10.0
P-36[m]	0.36	0.25	>100	11.1	10.7
KB[n]	1.4	1.4	>100	30.5	12.1
KATO III[o]	8.3	8.3	>100	>100	3.7
SW-480[p]	3.8	4.4	>100	>100	3.3
TE-2[q]	2.9	1.8	>100	>100	3.9
T-24[r]	1.1	0.94	>100	0.5	6.1

[a] See Table I for antineoplastic activity assay in vitro. Each tumor cell line (1 × 10^4/well) was incubated in the presence or absence of compounds for 72 h. [b] IC_{50}, μg/mL, was given as the concentration at 50% inhibition of cell growth. [c] Mouse melanoma. [d] Mouse Lewis lung carcinoma. [e] Human T-cell acute lymphoblastoid leukemia. [f] Human T-cell acute lymphoblastic leukemia. [g] Human promyelotic leukemia. [h] Human histiocytic lymphoma. [i] Human lung small-cell carcinoma. [j] Human lung squamus cell carcinoma. [k] Human lung large-cell carcinoma. [l] Human lung adenocarcinoma. [m] Human melanoma. [n] Human oral epidermoid carcinoma. [o] Human gastric carcinoma. [p] Human colon adenocarcinoma. [q] Human esophagus adenocarcinoma. [r] Human bladder transitional-cell carcinoma.

nocarcinoma and carcinoma cells except P36, KB, and T24 cells. By contrast, 5-FU had a broad spectrum of activity on this range of tumor cells, except for PC10 cells. Although DMDC is an analogue of 2'-deoxycytidine, its spectrum of activity against tumor cells is quite different from that of ara-C. DMDC is highly active against not only leukemia and lymphoma cell lines but also adenocarcinoma and carcinoma cell lines. The antineoplastic spectrum of DMDC is rather similar to that of Cytarazid[14] and 5-FU, but in some of the cell lines such as P36, KB, and T24, DMDC is more active than 5-FU. As can be seen from Table II, FDMDC has a quite similar spectrum to that of DMDC.

Preliminary in vivo antitumor activity of DMDC was also examined by using female $CD2F_1$ mice bearing ip inoculated L1210 leukemia. DMDC administered ip once a day for 5 days at 250 mg/kg beginning 24 h after tumor inoculation had a T/C (%) of 235. The activity of DMDC was schedule-dependent with much more therapeutic effect obtained by daily treatment than by a single treatment. DMDC also had therapeutic activity against human tumor xenografts, which will be reported elsewhere.[29] Thus, DMDC is an interesting and promising agent which should be considered for further detailed preclinical evaluation.

The detailed mechanism of action of DMDC and FDMDC is a future subject.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a JEOL JNM-FX 100 (100 MHz) or JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane as internal standard. ^{31}P NMR spectra were recorded on a JEOL FX90Q spectrometer with triethyl phosphate as internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was YMC gel 60A (70–230 mesh).

4-Ethoxy-1-[2-deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-2(1*H*)-pyrimidinone (4). Butyllithium (1.59 M solution in hexane, 3.15 mL, 5 mmol) was added to a suspension of methyltriphenylphosphonium bromide (2.15 g, 6 mmol) in dry THF (30 mL) with stirring for 10 min at 0 °C. A solution of **3**[22] (512 mg, 1 mmol) in THF (10 mL) was added dropwise to the above ylide solution at 0 °C and the reaction mixture was further stirred for 2 h at room temperature. Aqueous ammonium bromide solution (1 N, 10 mL) was added to the mixture, and the whole was extracted with EtOAc (50 mL), which was washed with H_2O (50 mL × 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography on silica gel (2.4 × 15 cm) with 20% EtOAc in hexane, giving 4 (210 mg, 41%) as a foam. The column was further eluted with 7% EtOH in $CHCl_3$ affording the phosphonium salt (**5**, 437 mg, 51% as a crude foam). Data for 4: MS *m/z* 510 (M^+); ^{1}H NMR ($CDCl_3$) 1.04 (28 H, m, isopropyl), 1.35 (3 H, t, 4-OEt, *J* = 7.1 Hz), 3.70 (1 H, dt, H-4', *J* = 2.5, 9.0 Hz), 4.11 (2 H, m, H-5'a, b), 4.43 (2 H, q, 4-OEt, *J* = 7.1 Hz), 4.78 (1 H, m, H-3'), 5.39 (1 H, dd, H-2''a, *J* = 1.5, 2.9 Hz), 5.69 (1 H, dd, H-2''b, *J* = 1.2, 2.9 Hz), 5.83 (1 H, d, H-5, *J* = 7.3 Hz), 6.64 (1 H, br d, H-1', *J* = 1.5 Hz), 7.76 (1 H, d, H-6, *J* = 7.3 Hz). Data for **5**: ^{1}H NMR ($CDCl_3$) 0.85–1.11 (28 H, m, isopropyl), 1.37 (3 H, t, 4-OEt, *J* = 7.1 Hz), 3.88–5.04 (9 H, m, 2'-OH, H-2''a,b,3',4',5'a,b and 4-OEt), 5.75 (1 H, d, H-5, *J* = 7.3 Hz), 6.11 (1 H, s, H-1'), 7.50–7.78 (15 H, m, Ph), 8.20 (1 H, d, H-6, *J* = 7.3 Hz); ^{31}P NMR ($CDCl_3$) 23.44.

Conversion of 5 into 4. NaH (60% in mineral oil, 200 mg, 5 mmol) was added to a solution of **5** (428 mg, 0.5 mmol) in THF (10 mL). The mixture was stirred for 2 h at room temperature. Aqueous ammonium bromide solution (1 M, 20 mL) was added to the mixture, and the whole was extracted with EtOAc (20 mL), which was washed with H_2O (10 mL × 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography on silica gel (2.4 × 11 cm) with 20% EtOAc in hexane, giving **4** (195 mg, 77%) as a foam.

4-Ethoxy-(2-deoxy-2'-methylidene-β-D-ribofuranosyl)-2-(1*H*)-pyrimidinone (6). A solution of TBAF (1 M THF solution, 1.5 mL, 1.5 mmol) was added to a solution of 4 (320 mg, 0.63 mmol) in THF (10 mL). The reaction mixture was stirred for 10 min at room temperature and then neutralized with AcOH. The solvent was removed in vacuo, and the residue was purified by a silica gel column (2.4 × 12 cm), which was eluted with 10% EtOH in $CHCl_3$ to afford **6** (154 mg, 91%) as a foam: MS *m/z* 268 (M^+); ^{1}H NMR ($CDCl_3$) 1.36 (3 H, t, 4-OEt, *J* = 7.3 Hz), 3.89 (3 H, m, H-4',5'a,b), 4.02 (1 H, dd, H-3', *J* = 2.9, 12.4 Hz), 4.43 (2 H, q, 4-OEt, *J* = 7.3 Hz), 4.88 (1 H, br s, 3'-OH), 5.45 (1 H, t, H-2''a, *J* = 2.2 Hz), 5.55 (1 H, t, H-2''b, *J* = 2.2 Hz), 5.89 (1 H, d, H-5, *J* = 7.3 Hz), 6.61 (1 H, d, H-1', *J* = 1.8 Hz), 7.60 (1 H, d, H-6, *J* = 7.3 Hz).

***N*^{4}-Benzoyl-1-[2-deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]cytosine (9). (a)** Butyllithium (1.59 M solution in hexane, 15.8 mL, 25 mmol) was added to a suspension of methyltriphenylphosphonium bromide (10.7 g, 30 mmol) in dry THF (60 mL) with stirring for 1 h at −20 °C. A solution of **8** (2.94 g, 5 mmol) in THF (20 mL) was added dropwise to the above ylide solution at 0 °C, and the reaction mixture was further stirred for 2 h at room temperature. Aqueous ammonium bromide solution (1 M, 50 mL) was added to the mixture, and the whole was extracted with EtOAc (200 mL), which was washed with H_2O (50 mL × 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography on silica gel (2.4 × 20 cm) with 25% EtOAc in hexane, giving **9** (907 mg, 31%) as a foam. The column was successively eluted with 7% EtOH in $CHCl_3$,

(29) Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T.; Sasaki, T. *Cancer Res.*, submitted for publication.

Table III. DMDC and Its Derivatives[a]

compd	yield, %[b]	mp, °C	crystn solvent	MS m/z (M^+)	formula
DMDC·HCl	81	>300	MeOH/acetone		$C_{10}H_{13}N_3O_4$·HCl
19a	86	185–187	EtOH/EtOAc	257	$C_{10}H_{12}FN_3O_4$
19b	93	168–170	EtOH/EtOAc	273	$C_{10}H_{12}ClN_3O_4$
19c	98	172–180 dec	EtOH/EtOAc	318, 320	$C_{10}H_{12}BrN_3O_4$
19d	97	foam		365	$C_{10}H_{12}IN_3O_4$
19e	89	198–204 dec	EtOH/EtOAc	253	$C_{11}H_{15}N_3O_4$
19g	85	194–196	EtOH/EtOAc	267	$C_{12}H_{17}N_3O_4$
16h	80	163–165	EtOH/hexane	240	$C_{10}H_{12}N_2O_5$
16a	81	154–156	EtOH/hexane	258	$C_{10}H_{11}FN_2O_5$
16b	91	149–152	EtOH/hexane	274	$C_{10}H_{11}ClN_2O_5$
16c	86	161–163	EtOH/hexane	319, 321	$C_{10}H_{11}BrN_2O_5$
16d	66	169–172	EtOH/hexane	366	$C_{10}H_{11}IN_2O_5$
16e	85	161–162	EtOH/hexane	254	$C_{11}H_{14}N_2O_5$
16f	87	172–174	EtOH/hexane	264	$C_{12}H_{12}N_2O_5$
16g	87	137–139	EtOH/hexane	268	$C_{12}H_{16}N_2O_5$

[a] All compounds in Table III gave satisfactory elemental analyses. [b] Yields were given at the deblocking step.

Table IV. ^{1}H NMR Parameters for 2′-Deoxy-2′-methylideneuridines and -cytidines: Chemical Shifts (δ) (in DMSO-d_6 with D_2O)

compd	H-1′	H-2″a,b	H-3′	H-4′,5′a,b	H-6	others
DMDC	6.52 br s	5.14 br s, 5.30 br s	4.40 m	3.40–3.90 m	7.48 d[a]	5.88 d (H-5)[a]
FDMDC	6.47 br s	5.22 br s, 5.32 br s	4.51 m	3.60–3.80 m	7.83 d[b]	
19b	6.48 br s	5.22 br s, 5.31 br s	4.51 m	3.50–3.80 m	7.82 s	
19c	6.45 br s	5.23 br s, 5.33 br s	4.50 m	3.61–3.70 m	7.79 s	
19d	6.45 br s	5.19 br s, 5.32 br s	4.51 m	3.50–3.75 m	7.83 s	
19e	6.52 br s	5.12 br s, 5.29 br s	4.47 m	3.50–3.80 m	7.30 s	1.81 s (5-Me)
19g	6.53 br s	5.15 br s, 5.29 br s	4.50 m	3.50–3.80 m	7.30 s	1.11 t, 2.22 q (5-Et)[c]
16h	6.42 d[d]	5.22 br s, 5.37 br s	4.49 m	3.56–3.63 m	7.50 d[e]	5.61 d (H-5)[e]
16a	6.44 t[f]	5.31 br s, 5.40 br s	4.52 m	3.57–3.74 m	7.95 d[g]	
16b	6.44 d[h]	5.33 br s, 5.40 br s	4.55 m	3.50–3.98 m	8.04 s	
16c	6.43 d[h]	5.33 br s, 5.40 br s	4.52 m	3.52–3.82 m	8.05 s	
16d	6.41 d[i]	5.30 br s, 5.39 br s	4.53 m	3.59–3.69 m	8.09 s	
16e	6.44 d[j]	5.23 br s, 5.39 br s	4.49 m	3.52–3.68 m	7.36 d	1.75 d (5-Me)
16f	6.52 br s	5.22 br s, 5.38 br s	4.48 m	3.50–3.80 m	8.35 s	3.22 s (5-C≡CH)
16g	6.76 d[j]	5.24 br s, 5.38 br s	4.52 m	3.50–3.80 m	7.34 s	1.00 t, 2.20 q (5-Et)

[a] $J_{5,6}$ = 7.5 Hz. [b] $J_{F,6}$ = 7.1 Hz. [c] J = 7.3 Hz. [d] $J_{1',2''}$ = 1.5 Hz. [e] $J_{5,6}$ = 8.1 Hz. [f] $J_{F,1'} = J_{1',2''}$ = 1.7 Hz. [g] $J_{F,6}$ = 6.6 Hz. [h] $J_{1',2''}$ = 1.3 Hz. [i] $J_{1',2''}$ = 1.1 Hz. [j] $J_{1',2''}$ = 1.5 Hz.

affording the phosphonium salt (10, 3.67 g, 77% as a crude foam). Data for 9: MS m/z 585 (M^+); ^{1}H NMR ($CDCl_3$) 1.01–1.13 (28 H, m, isopropyl), 3.75 (1 H, dt, H-4′, $J_{3',4'}$ = 9.0, $J_{4',5'}$ = 2.4 Hz), 4.02–4.33 (2 H, m, H-5′a,b), 4.80 (1 H, ddd, H-3′, $J_{2',3'}$ = 1.2, $J_{3',4'}$ = 9.0 Hz), 5.43 (1 H, dd, H-2″a, J = 1.0, 1.7 Hz), 5.79 (1 H, dd, H-2″b, J = 1.0, 1.2 Hz), 6.66 (1 H, dd, H-1′, J = 1.2 Hz), 7.42–7.91 (6 H, m, H-5, Ph), 8.06 (1 H, d, H-6, J = 7.6 Hz), 8.70 (1 H, br s, 4-NH). Data for 10: ^{1}H NMR ($CDCl_3$) 0.89–1.11 (28 H, m, isopropyl), 3.77–4.95 (7 H, m, 2′-OH, H-2″a,b,3′,4′,5′a,b), 6.23 (1 H, s, H-1′), 7.53–8.01 (16 H, m, H-5, Ph), 8.28 (1 H, d, H-6, $J_{5,6}$ = 7.6 Hz), 8.40 (1 H, br s, 4-NH); ^{31}P NMR ($CDCl_3$) 23.45. Compound 10 (478 mg, 0.5 mmol) was similarly converted as described in conversion of 5 to yield 9 (152 mg, 52%) after column chromatographic purification.

(b) Butyllithium (1.59 M solution in hexane, 7.6 mL, 12 mmol) was added to a suspension of methyltriphenylphosphonium bromide (4.29 g, 12 mmol) in dry THF (40 mL) with stirring for 1 h at 0 °C. A solution of 8 (1.76 g, 3 mmol) in THF (15 mL) was added dropwise to the above ylide solution at 0 °C, and the reaction mixture was further stirred for 4 h at room temperature. Aqueous ammonium bromide solution (1 M, 40 mL) was added to the mixture, and the whole was extracted with EtOAc (200 mL), which was washed with H_2O (30 mL × 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was dissolved in THF (40 mL) in which NaH (60% in mineral oil, 480 mg, 12 mmol) was added. The mixture was stirred for 3 h at room temperature, and the reaction was quenched by addition of aqueous ammonium bromide solution (1 M, 30 mL). The mixture was extracted with EtOAc (100 mL), and the separated organic phase was washed with H_2O (20 mL × 2), dried (Na_2SO_4), and concentrated to dryness. The residue was purified by column chromatography on silica gel (2.4 × 23 cm) with 25% EtOAc in hexane, giving 9 (1.41 g, 80%).

N^4-Benzoyl-2′-deoxy-2′-methylidenecytidine (11). A solution of TBAF (1 M THF solution, 2.2 mL, 2.2 mmol) was added to a solution of 9 (343 mg, 1 mmol) in THF (10 mL). The reaction mixture was stirred for 30 min at room temperature and then neutralized with AcOH. The solvent was removed in vacuo and the residue was purified by a silica gel column (1.6 × 13 cm), which was eluted with 8% EtOH in $CHCl_3$ to afford 11 (302 mg, 88%; crystallized from EtOH–Et_2O): mp > 300 °C; MS m/z 343 (M^+); ^{1}H NMR ($CDCl_3$) 3.42–3.71 (3 H, m, H-4′, 5′a,b), 4.56 (1 H, m, H-3′), 5.06 (1 H, t, 5′-OH), 5.38 (2 H, br s, H-2″a,b), 5.70 (1 H, d, 3′-OH), 6.61 (1 H, br s, H-1′), 7.32–8.05 (7 H, m, H-5, 4-NH, Ph), 8.19 (1 H, d, H-6, $J_{5,6}$ = 7.3 Hz). Anal. ($C_{17}H_{17}N_3O_5$) C, H, N.

2′-Deoxy-2′-methylidenecytidine Hydrochloride (DMDC Hydrochloride). (a) A solution of 6 (150 mg, 0.56 mmol) in methanolic ammonia (saturated at 0 °C, 10 mL) in a sealed stainless container was heated for 48 h at 100 °C. The container was cooled at room temperature and degassed. The mixture was concentrated in vacuo, and the residue was dissolved in 1 N HCl (1 mL), which was evaporated and coevaporated several times with EtOH. The residue was crystallized from MeOH–acetone to give DMDC (125 mg, 81%) as a hydrochloride: mp > 300 °C; see Tables III and IV for other physical properties.

(b) A solution of 11 (139 mg, 0.5 mmol) in methanolic ammonia (saturated at 0 °C, 10 mL) was kept for 6 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in 1 N HCl (1 mL) which was evaporated and coevaporated several times with EtOH. The residue was crystallized as described above to afford DMDC (115 mg, 83%) as a hydrochloride.

General Procedure for Selective Protection of the 3′,5′-Hydroxyl Group in 12a–f,h. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDSCl_2, 1.05 mol equiv) was added to a solution of the nucleoside 12 in dry pyridine at 0 °C. The mixture was stirred for several hours at room temperature, and the reaction was quenched by the addition of ice–water. The mixture was concentrated to dryness in vacuo, and the residue was partitioned between EtOAc and H_2O. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness, and the residue was chromatographed over a silica gel column with 20–25% EtOAc

in hexane. This gave the corresponding 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-substituted uracil **13a–f,h**.

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-fluorouracil (15a). A mixture of **12a** (2.62 g, 10 mmol) and $TIPDSCl_2$ (3.3 mL, 10.5 mmol) in pyridine (30 mL) was stirred for 3.5 h at room temperature. After addition of ice–water, the solvent was removed in vacuo and the residue was partitioned between EtOAc (100 mL) and H_2O (50 mL × 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by a silica gel column (2.4 × 23 cm), eluted with 25% EtOAc in hexane, to give 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-fluorouracil (**13a**, 4.24 g, 84% as a foam): MS *m/z* 504 (M^+); ^1H NMR ($CDCl_3$) 1.02–1.11 (28 H, m, isopropyl), 3.34 (1 H, s, 2'-OH), 3.93–4.31 (5 H, m, H-2',3',4',5'a,b), 5.76 (1 H, s, H-1'), 7.87 (1 H, d, H-6, $J_{F,6}$ = 6.1 Hz), 9.40 (1 H, br s, 3-NH). A solution of **13a** (3.91 g, 7.8 mmol) in CH_2Cl_2 (10 mL) was added to a preformed oxidation complex [CrO_3 (3.0 g), pyridine (5 mL), and Ac_2O (3 mL) in CH_2Cl_2 (80 mL)]. The reaction mixture was stirred for 1 h at room temperature and was poured dropwise to EtOAc (600 mL). The suspension was filtered through a short silica gel column (6 × 1.5 cm) and the filtrate was concentrated to dryness. The residue was purified by a silica gel column (2.4 × 23 cm) with 20% EtOAc in hexane to afford **14a** (2.8 g, 72%, crystallized from hexane): mp 200–202 °C; MS *m/z* 502 (M^+); ^1H NMR ($CDCl_3$) 1.04–1.11 (28 H, m, isopropyl), 3.89–4.15 (3 H, m, H-4', 5'a,b), 4.97 (1 H, s, H-1'), 5.01 (1 H, d, H-3', *J* = 8.1 Hz), 7.24 (1 H, d, H-6, *J* = Hz), 8.49 (1 H, br s, 3-NH). Anal. ($C_{21}H_{35}FN_2O_7Si_2$) C, H, N. A suspension of KH (1.2 g, 29.8 mmol, washed with dry hexane under argon) in dimethyl sulfoxide (DMSO, 12 mL) was stirred for 1 h at room temperature under argon. A solution of methyltriphenylphosphonium bromide (12 g, 33.6 mmol) in dry DMSO (25 mL) was added to the above suspension at room temperature. The mixture was stirred for 10 min then cooled at 0 °C. A solution of **14a** (1.5 g, 2.98 mmol) in DMSO (10 mL) was added dropwise to the ylide solution and the whole was stirred for 1 h at 0 °C. The reaction was quenched by addition of aqueous ammonium bromide solution (1 M, 20 mL) which was diluted with EtOAc (100 mL) and washed with H_2O (50 mL × 3). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was chromatographed over a silica gel column (2.4 × 22 cm) with 17% EtOAc in hexane to give **15a** (980 mg, 66%): MS *m/z* 500 (M^+); ^1H NMR ($CDCl_3$) 1.05–1.10 (28 H, m, isopropyl), 3.69 (1 H, br d, H-4', *J* = 8.8 Hz), 3.94–4.14 (2 H, m, H-5'a,b), 4.80 (1 H, m, H-3'), 5.45–5.61 (2 H, m, H-2''-a,b), 6.50 (1 H, dd, H-1', *J* = 1.2, 1.5 Hz), 7.58 (1 H, d, H-6, *J* = 6.1 Hz), 8.58 (1 H, br s, 3-NH).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-chlorouracil (15b). Reaction of **12b** (10 g, 35.9 mmol) and $TIPDSCl_2$ (12.44 mL, 39.5 mmol) in pyridine (100 mL) for 5 h at room temperature gave 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-chlorouracil (**13b**, 14.6 g, 78%, as a foam) after purification by a silica gel column: MS *m/z* 478 (M^+ – isopropyl); ^1H NMR ($CDCl_3$) 1.03–1.26 (28 H, m, isopropyl), 3.44 (1 H, s, 2'-OH), 3.92–4.33 (5 H, m, H-2',3,4',5'a,b), 5.74 (1 H, s, H-1'), 7.96 (1 H, s, H-6), 9.55 (1 H, br s, 3-NH). Oxidation of **13b** (5.21 g, 10 mmol) with the chromium complex [CrO_3 (4 g), pyridine (6.6 mL), and Ac_2O (4 mL) in CH_2Cl_2 (100 mL)] was done for 2 h at room temperature. After purification by a silica gel column, 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]-5-chlorouracil (**14b**, 3.75 g, 72%, crystallized from hexane) was obtained: mp 199–201 °C; MS *m/z* 476 (M^+ – isopropyl); ^1H NMR ($CDCl_3$) 1.03–1.11 (28 H, m, isopropyl), 3.85–4.14 (3 H, m, H-4', 5'a,b), 5.01 (1 H, s, H-1'), 5.02 (1 H, d, H-3', *J* = 8.4 Hz), 7.63 (1 H, s, H-6), 8.36 (1 H, br s, 3-NH). Anal. ($C_{21}H_{35}ClN_2O_7Si_2$) C, H, N. Treatment of **14b** (2.35 g, 4.5 mmol) with methylenetriphenylphosphorane (31.7 mmol, prepared from 12.9 g of triphenylphosphonium bromide and 31.7 mmol of BuLi in 50 mL of THF for 10 min at 0 °C) for 3 h at room temperature gave **15b** (1.12 g, 48%, as a foam): MS *m/z* 517 (M^+); ^1H NMR ($CDCl_3$) 0.94–1.10 (28 H, m, isopropyl), 3.70 (1 H, ddd, H-4', *J* = 8.8 Hz), 4.11 (2 H, m, H-5'a,b), 4.82 (1 H, m, H-3'), 5.48 (1 H, dd, H-2''a), 5.57 (1 H, dd, H-2''b, *J* = 1.5 Hz), 6.50 (1 H, br d, H-1', *J* = 1.0 Hz), 7.64 (1 H, s, H-6), 8.83 (1 H, br s, 3-NH).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-bromouracil (15c). Treatment of **12c** (3.32 g, 10 mmol) and $TIPDSCl_2$ (3.3 mL, 10.5 mmol) in pyridine (30 mL) for 4 h at room temperature afforded 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-bromouracil (5.05 g, 89%, as a foam): MS *m/z* 522 (M^+ – isopropyl); ^1H NMR ($CDCl_3$) 1.04–1.26 (28 H, m, isopropyl), 3.55 (1 H, s, 2'-OH), 3.93–4.32 (5 H, m, H-2',3',4',5'a,b), 5.73 (1 H, s, H-1'), 7.98 (1 H, s, H-6), 9.64 (1 H, br s, 3-NH). Oxidation of **13c** (4.3 g, 7.6 mmol) with the chromium complex (4 equiv in 80 mL of CH_2Cl_2) for 2 h at room temperature gave 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]-5-bromouracil (**14c**, 2.4 g, 56%, as a foam) after purification by a silica gel column: MS *m/z* 520 (M^+ – isopropyl); ^1H NMR ($CDCl_3$) 1.03–1.11 (28 H, m, isopropyl), 3.85–4.14 (3 H, m, H-4',5'a,b), 5.01 (1 H, s, H-1'), 5.02 (1 H, d, H-3', *J* = 8.4 Hz), 7.78 (1 H, s, H-6), 8.25 (1 H, br s, 3-NH). Reaction of **14c** (650 mg, 1.15 mmol) with methylenetriphenylphosphorane (7.9 mmol) in THF (60 mL) for 4 h under the above conditions gave **15c** (380 mg, 59%, as a foam): MS *m/z* 561 (M^+); ^1H NMR ($CDCl_3$) 1.06–1.11 (28 H, m, isopropyl), 3.69 (1 H, br d, H-4', *J* = 8.5 Hz), 3.89–4.13 (2 H, m, H-5'a,b), 4.78–4.88 (1 H, m, H-3'), 5.49–5.98 (2 H, m, H-2''a,b), 6.50 (1 H, br s, H-1'), 7.71 (1 H, s, H-6), 8.49 (1 H, br s, 3-NH).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-iodouracil (15d). Compound **12d** (10 g, 27 mmol) was treated with $TIPDSCl_2$ (8.9 mL, 28.4 mmol) in pyridine (100 mL) for 4.5 h at room temperature. After the usual workup and purification this afforded 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-iodouracil (**13d**, 14.7 g, 89%, as a foam): MS *m/z* 612 (M^+); ^1H NMR ($CDCl_3$) 1.04–1.11 (28 H, m, isopropyl), 3.54 (1 H, br s, 2'-OH), 3.93–4.41 (5 H, m, H-2',3',4',5'a,b), 5.73 (1 H, s, H-1'), 7.99 (1 H, s, H-6), 8.88 (1 H, br s, 3-NH). Oxidation of **13d** (13.65 g, 22.3 mmol) with the chromium complex (4 equiv in 230 mL of CH_2Cl_2) for 2 h at room temperature gave 4.4 g of 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]-5-iodouracil (**14d**, 32%, as a foam): MS *m/z* 610 (M^+); ^1H NMR ($CDCl_3$) 1.03–1.11 (28 H, m, isopropyl), 3.84–4.14 (3 H, m, H-4',5'a,b), 5.00 (1 H, s, H-1'), 5.02 (1 H, d, H-3', *J* = 8.4 Hz), 7.51 (1 H, s, H-6), 8.64 (1 H, br s, 3-NH). Reaction of **14d** (4.0 g, 6.55 mmol) with methylenetriphenylphosphorane (32.8 mmol) in THF (100 mL) for 4 h at room temperature gave 960 mg of **15d** (24%, as a foam): MS *m/z* 608 (M^+); ^1H NMR ($CDCl_3$) 1.02–1.14 (28 H, m, isopropyl), 3.68 (1 H, dt, H-4', *J* = 2.2, 8.8 Hz), 3.90–4.13 (2 H, m, H-5'a,b), 4.83 (1 H, dd, H-3', *J* = 1.7, 9.0 Hz), 5.47–5.57 (2 H, m, H-2''a,b), 6.49 (1 H, d, H-1', *J* = 1.5 Hz), 7.75 (1 H, s, H-6), 8.88 (1 H, br s, 3-NH).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-methyluracil (15e). Compound **12e** (7.75 g, 30 mmol) was treated with $TIPDSCl_2$ (10.44 mL, 33 mmol) in pyridine (90 mL) for 6 h at room temperature to give 1-[3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-β-D-ribofuranosyl)-5-methyluracil (**13e**, 15.0 g, 98%, as a foam): MS *m/z* 500 (M^+); ^1H NMR ($CDCl_3$) 1.08 (28 H, m, isopropyl), 1.91 (3 H, d, 5-Me, *J* = 1.0 Hz), 3.17 (1 H, d, 2'-OH, *J* = 1.7 Hz), 4.13 (4 H, m, H-2',4',5'a,b), 4.40 (1 H, dd, H-3', *J* = 5.1, 8.1 Hz), 5.71 (1 H, s, H-1'), 7.38 (1 H, d, H-6, *J* = 1.0 Hz), 8.67 (1 H, br s, 3-NH). Dimethyl sulfoxide (3 mL, 45 mmol) was added dropwise over 10 min to a solution of oxalyl chloride (1.7 mL, 21 mmol) in CH_2Cl_2 (40 mL) at −78 °C under argon. To this mixture, a solution of **13e** (8.04 g, 16.1 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 20 min. The whole was stirred for a further 1 h, then triethylamine (13.5 mL) was added at once. After being warmed to room temperature, the mixture was washed with H_2O (20 mL × 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by a silica gel column gave 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]-5-methyluracil (**14e**, 6.68 g, 83%). Crystallization of this nucleoside from hexane afforded an analytical sample: mp 168–170 °C; MS *m/z* 498 (M^+); ^1H NMR ($CDCl_3$) 1.11 (28 H, m, isopropyl), 1.91 (3 H, d, 5-Me, *J* = 1.2 Hz), 4.10 (3 H, m, H-4',5'a,b), 4.98 (1 H, s, H-1'), 5.04 (1 H, d, H-3', *J* = 9.3 Hz), 6.96 (1 H, d, H-6, *J* = 1.2 Hz), 8.06 (1 H, br s, 3-NH). Anal. ($C_{22}H_{38}N_2O_7Si_2$) C, H, N. Reaction of **14e** (2.0 g, 4 mmol) with methylenetriphenylphosphorane (10 mmol) in THF (20 mL) for 2 h at room temperature gave 397 mg of **15e** (40%, as a foam):

MS m/z 496 (M^+); ^1H NMR ($CDCl_3$) 1.10 (28 H, m, isopropyl), 1.89 (3 H, d, 5-Me, J = 1.1 Hz), 3.66 (1 H, ddd, H-4′, J = 2.2, 2.9, 8.8 Hz), 4.05 (1 H, dd, H-5′a, J = 2.9, 13.2 Hz), 4.13 (1 H, dd, H-5′b, J = 2.2, 13.2 Hz), 4.86 (1 H, dd, H-3′, J = 1.8, 8.8 Hz), 5.47 (2 H, m, H-2″a,b), 6.54 (1 H, dd, H-1′, J = 1.5, 3.3 Hz), 7.10 (1 H, d, H-6, J = 1.1 Hz), 8.41 (1 H, br s, 3-NH).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-ethynyluracil (15f). Reaction of **12f**[30] (2.1 g, 7.8 mmol) with $TIPDSCl_2$ (2.6 mL, 8.2 mmol) in pyridine (50 mL) for 15 h at room temperature afforded 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-ethynyluracil (**13f**, 3.26 g, 82%, as a foam): MS m/z 510 (M^+); ^1H NMR ($CDCl_3$) 1.10 (28 H, m, isopropyl), 3.09 (1 H, d, 2′-OH, J = 1.7 Hz), 3.17 (1 H, s, acetylene proton), 3.93–4.36 (5 H, m, H-2′,3′,4′,5′a,b), 5.74 (1 H, s, H-1′), 7.95 (1 H, s, H-6), 8.75 (1 H, br s, 3-NH). Oxidation of **13f** (2.04 g, 10 mmol) with the chromium complex (4 equiv in 30 mL of CH_2Cl_2) gave **14f** (1.62 g, 81%, as a foam): MS m/z 508 (M^+); ^1H NMR ($CDCl_3$) 1.12 (28 H, m, isopropyl), 3.16 (1 H, s, acetylene proton), 3.91 (1 H, m, H-4′), 4.13 (2 H, m, H-5′a,b), 5.04 (1 H, s, H-1′), 5.10 (1 H, d, H-3′, J = 9.0 Hz), 7.31 (1 H, s, H-6), 8.20 (1 H, br s, 3-NH). Reaction of **14f** (1.52 g, 3 mmol) with methylenetriphenylphosphorane (12 mmol) in THF (40 mL) for 6 h at room temperature gave **15f** (1.12 g, 74%, as a foam): MS m/z 506 (M^+); ^1H NMR ($CDCl_3$) 1.11 (28 H, m, isopropyl), 3.17 (1 H, s, acetylene proton), 3.71 (1 H, br d, H-4′, J = 9.1 Hz), 4.10 (2 H, m, H-5′a,b), 4.85 (1 H, m, H-3′), 5.48 (2 H, m, H-2″a,b), 6.48 (1 H, br d, H-1′, J = 1.5 Hz), 7.88 (1 H, s, H-6), 8.41 (1 H, br s, 3-NH).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-ethyluracil (15g). A suspension of 10% Pd/C (100 mg) and **13f** (3.8 g, 7.43 mmol) in MeOH (50 mL) was shaken under H_2 atmosphere (20 psi) for 4 h at room temperature. The mixture was filtered by a Celite pad and the filtrate was concentrated dryness in vacuo. The residue was purified by a silica gel column (3.0 × 20 cm) eluted with 30% EtOAc in hexane to afford 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-ethyluracil (**13g**, 3.22 g, 84% as a foam): MS m/z 514 (M^+); ^1H NMR ($CDCl_3$) 1.12 (31 H, m, isopropyl, 5-CH_2CH_3), 2.34 (2 H, q, 5-CH_2CH_3), 3.19 (1 H, d, 2′-OH, J = 1.7 Hz), 3.95–4.54 (5 H, m, H-2′,3′,4′,5′a,b), 5.67 (1 H, d, H-1′, $J_{1',2'}$ = 1.2 Hz), 7.29 (1 H, s, H-6), 8.66 (1 H, br s, 3-NH). Oxidation of **13g** (3.15 g, 6.12 mmol) with the chromium complex (4 equiv in 50 mL of CH_2Cl_2) gave 1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]-5-ethyluracil (**14g**, 2.54 g, 81%, crystallized from EtOAc/hexane): mp 176–177 °C; MS m/z 512 (M^+); ^1H NMR ($CDCl_3$) 1.15 (31 H, m, isopropyl, 5-CH_2CH_3), 2.35 (2 H, q, 5-CH_2CH_3, J = 7.6 Hz), 3.90 (1 H, dt, H-4′, J = 3.4, 9.0 Hz), 4.11 (2 H, m, H-5′a,b), 4.98 (1 H, s, H-1′), 5.07 (1 H, d, H-3′, J = 9.0 Hz), 6.90 (1 H, s, H-6), 8.01 (1 H, br s, 3-NH). Anal. ($C_{23}H_{40}N_2O_7Si_2$) C, H, N. Reaction of **14g** (2.05 g, 4 mmol) with methylenetriphenylphosphorane (16 mmol) in THF (50 mL) for 6 h at room temperature gave 1.70 g of **15g** (83%, crystallized from EtOAc/hexane): mp 153–155 °C; MS m/z 510 (M^+); ^1H NMR ($CDCl_3$) 1.10 (31 H, m, isopropyl, 5-CH_2CH_3), 2.32 (2 H, q, 5-CH_2CH_3), 3.66 (1 H, dt, H-4′, J = 2.7, 9.0 Hz), 4.09 (2 H, m, H-5′a,b), 4.88 (1 H, ddd, H-3′, J = 2.7, 2.9, 9.0 Hz), 5.47 (2 H, m, H-2″a,b), 6.54 (1 H, br d, H-1′, J = 1.7 Hz), 7.02 (1 H, s, H-6), 8.10 (1 H, br s, 3-NH). Anal. ($C_{24}H_{42}N_2O_6Si_2$) C, H, N.

Conversion of Uracil Nucleosides (15) into Cytosine Nucleosides (18) via the 1,2,4-Triazolo Derivative. $POCl_3$ (620 μL, 6.67 mmol) was added to a solution of 1,2,4-triazole (1.52 g, 22 mmol) in dry CH_3CN (20 mL) containing Et_3N (3 mL, 22 mmol) at 0 °C under argon. The mixture was stirred for 1 h at room temperature, then the insoluble material was removed by filtration under argon. The filtrate was mixed with nucleoside (**15**, 2 mmol), and the mixture was stirred for 4 h at room temperature. NH_3 gas was bubbled into the mixture through the drying tube (NaOH) for an appropriate time as judged by TLC. The resulting mixture was diluted with $CHCl_3$ (100 mL) and was washed with H_2O (100 mL). The aqueous phase was reextracted with $CHCl_3$ (20 mL × 2), and the $CHCl_3$ layers were combined, dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified by a silica gel column, eluted with 10% EtOH in $CHCl_3$.

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-fluorocytosine (18a). From 500 mg (1 mmol) of **15a**, **18a** was obtained in 87% yield (435 mg, as a glass): MS m/z 499 (M^+); ^1H NMR ($CDCl_3$) 1.12 (28 H, m, isopropyl), 3.71 (1 H, dt, H-4′, J = 2.4, 8.8 Hz), 4.07 (1 H, dd, H-5′a, J = 2.4, 10.7 Hz), 4.15 (1 H, dd, H-5′b, J = 2.0, 10.7 Hz), 4.69 (1 H, ddd, H-3′, J = 2.7, 2.9, 8.8 Hz), 5.39 (1 H, dd, H-2″a, J = 1.7, 2.8 Hz), 5.70 (1 H, dd, H-2″b, J = 1.2, 2.8 Hz), 6.59 (1 H, br s, H-1′), 7.66 (1 H, d, H-6, J = 6.4 Hz).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-chlorocytosine (18b). From 1.04 g (2 mmol) of **15b**, **18b** was obtained in 91% yield (944 mg, crystallized from EtOAc): mp 108–111 °C; MS m/z 516 (M^+); ^1H NMR ($CDCl_3$) 1.10 (28 H, m, isopropyl), 3.65 (1 H, br d, H-4′, J = 8.6 Hz), 4.11 (2 H, m, H-5′a,b), 4.70 (1 H, br d, H-3′, J = 8.6 Hz), 5.40 (1 H, br s, H-2″a), 5.64 (1 H, br s, H-2″b), 6.59 (1 H, br s, H-1′), 7.70 (1 H, s, H-6), 7.82 (2 H, br s, 4-NH_2). Anal. ($C_{22}H_{38}ClN_3O_5Si_2$) C, H, N.

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-bromocytosine (18c). From 1.12 g (2 mmol) of **15c**, **18c** was obtained in 83% yield (626 mg, as a glass): MS m/z 560, 562 (M^+, M^+ + 2); ^1H NMR ($CDCl_3$) 1.20 (28 H, m, isopropyl), 3.66 (1 H, dt, H-4′, J = 2.4, 9.0 Hz), 4.10 (1 H, dd, H-5′a, J = 2.7, 13.4 Hz), 4.15 (1 H, dd, H-5′b, J = 1.9, 13.4 Hz), 4.81 (1 H, ddd, H-3′, J = 2.7, 2.9, 9.0 Hz), 5.40 (1 H, dd, H-2″a, J = 1.7, 2.8 Hz), 5.68 (1 H, dd, H-2″b, J = 1.0, 2.8 Hz), 6.60 (1 H, br s, H-1′), 7.75 (1 H, s, H-6), 8.35 (2 H, br s, 4-NH_2).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-iodocytosine (18d). From 321 mg (0.53 mmol) of **15d**, **18d** was obtained in 53% yield (169 mg, crystallized from EtOAc): mp 185–187 °C; MS m/z 608 (M^+); ^1H NMR ($CDCl_3$) 1.20 (28 H, m, isopropyl), 3.66 (1 H, dt, H-4′, J = 2.4, 9.0 Hz), 4.10 (1 H, dd, H-5′a, J = 2.7, 13.4 Hz), 4.15 (1 H, dd, H-5′b, J = 1.9, 13.4, Hz), 4.81 (1 H, ddd, H-3′, J = 2.7, 2.9, 9.0 Hz), 5.40 (1 H, dd, H-2″a, J = 1.7, 2.8 Hz), 5.68 (1 H, dd, H-2″b, J = 1.0, 2.8 Hz), 6.60 (1 H, br s, H-1′), 7.75 (1 H, s, H-6), 8.35 (2 H, br s, 4-NH_2). Anal. ($C_{22}H_{38}IN_3O_5Si_2$) C, H, N.

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-methylcytosine (18e). From 498 mg (1 mmol) of **15e**, **18e** was obtained in 63% yield (315 mg, as a glass): MS m/z 495 (M^+); ^1H NMR ($CDCl_3$) 1.11 (28 H, m, isopropyl), 1.99 (3 H, s, 5-Me), 3.67 (1 H, dt, H-4′, J = 2.2, 8.8 Hz), 4.11 (2 H, m, H-5′a,b), 4.82 (1 H, ddd, H-3′, J = 2.2, 2.9, 9.0 Hz), 5.37 (1 H, dd, H-2″a, J = 2.0, 2.8 Hz), 5.58 (1 H, dd, H-2″b, J = 1.5, 2.8 Hz), 6.71 (1 H, br s, H-1′), 7.26 (1 H, s, H-6), 8.35 (2 H, br s, 4-NH_2).

1-[2-Deoxy-2-methylidene-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-5-ethylcytosine (18g). From 1.2 g of **15g**, **18g** was obtained in 83% yield (1.0 g, crystallized from EtOAc/hexane): mp 197–200 °C; MS m/z 510 (M^+); ^1H NMR ($CDCl_3$) 1.05 (31 H, m, isopropyl, 5-CH_2CH_3), 2.28 (2 H, q, 5-CH_2CH_3), 3.66 (1 H, dt, H-4′, J = 2.4, 8.8 Hz), 4.10 (2 H, m, H-5′a,b), 4.82 (1 H, m, H-3′), 5.39 (1 H, dd, H-2″a, J = 1.7, 2.8 Hz), 5.55 (1 H, dd, H-2″b, J = 1.3, 2.8 Hz), 6.72 (1 H, dd, H-1′, J = 1.3, 1.7 Hz), 7.18 (1 H, s, H-6), 7.30 (2 H, br s, 4-NH_2). Anal. ($C_{24}H_{43}N_3O_5Si_2$) C, H, N.

General Procedure for Deprotection of 15 and 18. A THF solution of TBAF (1 M, 2.1 mol equiv) was added to a solution of **15** or **18** in dry THF (5 mL for 2 mmol of the nucleoside). The mixture was stirred for 30–60 min at room temperature and was quenched with AcOH. The solvent was removed in vacuo, and the residue was chromatographed over a silica gel column with 10–15% EtOH in $CHCl_3$ to give the corresponding free nucleoside. The yield, crystallization solvent, melting point, and MS spectral data of compounds **19a–e,g** and **16a–h** are listed in Table III. ^1H NMR spectral data of these nucleosides are summarized in Table IV. Compound **15h** was prepared by the reported method.[31]

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Structure–Stability Relationships of Gd(III) Ion Complexes for Magnetic Resonance Imaging

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Molecular mechanical calculations and molecular dynamics simulations, based on the AMBER force field, were used to examine the molecular structures and stabilities of nine multidentate ligands and their Gd(III) ion complexes. The magnitude of various factors determining the stability of multidentate Gd(III) complexes, including the energy loss due to change of ligand conformation by complexation, the energy gain from cation–ligand attraction, and effects of intramolecular hydrogen bonding, were calculated by molecular mechanics. The fit between the Gd cation and the binding cavity in the ligands was examined by molecular graphics techniques. Intramolecular hydrogen bonds in free ligands with amide or hydroxyl as H-bond donors usually disfavor complex formation, due to disruption of hydrogen bonds during complex formation. Intramolecular hydrogen bonds may contribute to enhance complex stability if they make the desolvation energy of the free ligands smaller. The calculated complex stabilities were in reasonable agreement with experimental log K values which were available for five of the compounds. The calculated complex stabilities of two hitherto unsynthesized covalently constrained DTPA-derivatives and a DOTA-derivative bearing phenoxy groups as pendant arms indicate that these may form Gd(III) complexes with sufficient stability for use in magnetic resonance imaging techniques.

Introduction

DTPA (1,4,7-triazaheptane-1,1,4,7,7-pentaacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate), and other aminopolycarboxylates form highly stable complexes with lanthanide ions.[1] Due to the high magnetic moment of the Gd(III) ion, Gd(III) complexes are widely used as contrast agents in magnetic resonance imaging techniques.[2] Complexation of the gadolinium ion is a prerequisite for diagnostic use since free lanthanide ions are highly toxic. It is important, therefore, that such complexes have high kinetic and thermodynamic stability, in order to prevent their dissociation in the body fluids.

The relationship between complex stability and molecular structure has previously been examined for the aminopolycarboxylate ligands DOTA, DTPA, DO3A (1,4,7,10-tetraazacyclododecane-N,N',N''-triacetic acid), and OTTA (oxa-4,7,10-triazacyclododecane-N,N',N'''-triacetic acid) and their Gd(III) complexes.[3] In the present study, the thermodynamic stabilities of the nine ligands shown in Chart I and their Gd(III) complexes were examined by molecular mechanical calculations and molecular dynamics simulations. One aim of the study was to obtain a model for computation of the stabilities of such complexes, and to evaluate the model from experimental thermodynamic log K values.

Five of the ligands shown in Chart I have previously been synthesized. These include DTPA-BMA (1,7-bis[(N-methylcarbamoyl)methyl]-1,4,7-triazaheptane-1,4,7-triacetic acid),[4] DTPA-HMA ((*S,S*)-2,6-bis(hydroxymethyl)-1,7-bis[(N-methylcarbamoyl)methyl]-1,4,7-triazaheptane-1,4,7-triacetic acid),[4] DTPA-BMPA (1,7-bis[[N-(2,3-dihydroxypropyl)-N-methylcarbamoyl]methyl]-1,4,7-triazaheptane-1,4,7-triacetic acid),[4] TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid),[5] and NOTA (1,4,7-triazacyclononane-N,N',N''-triacetic acid).[5] The other four compounds have not been synthesized. DTPA-TRANS (*trans*-2,6-bis[[N,N-bis(carboxymethyl)amino]methyl]piperidine-N-acetic acid), DTPA-CIS (*cis*-2,6-bis[[N,N-bis(carboxymethyl)amino]methyl]piperidine-N-acetic acid), PHEA (1,4,7-tris(2-hydroxyphenyl)-1,4,7,10-tetraazacyclododecane), and DTPA-HB (1,4,7-triazaheptane-1,1,4,7,7-pentakis[bis(hydroxy methyl)acetic acid]) were included as model compounds in order to examine structural effects on Gd(III) complex stabilities. DTPA-HB was included in the calculations in order to investigate effects of hydrogen bonding on complex stability. PHEA was included in order to investigate the ligating strength of phenoxy groups, and DTPA-CIS and DTPA-TRANS were included in order to compare the Gd(III) complex stabilities of these two isomers with that of DTPA.

The binding cavities in the NOTA, TETA, DOTA, and DTPA-HMA complexes were mapped by molecular graphics techniques, in order to investigate to what extent cation–cavity size fit determines the stability of Gd(III)

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