Synthesis and Anticancer and Antiviral Activities of Various 2′- and 3′-Methylidene-Substituted Nucleoside Analogues and Crystal Structure of 2′-Deoxy-2′-methylidenecytidine Hydrochloride[1]

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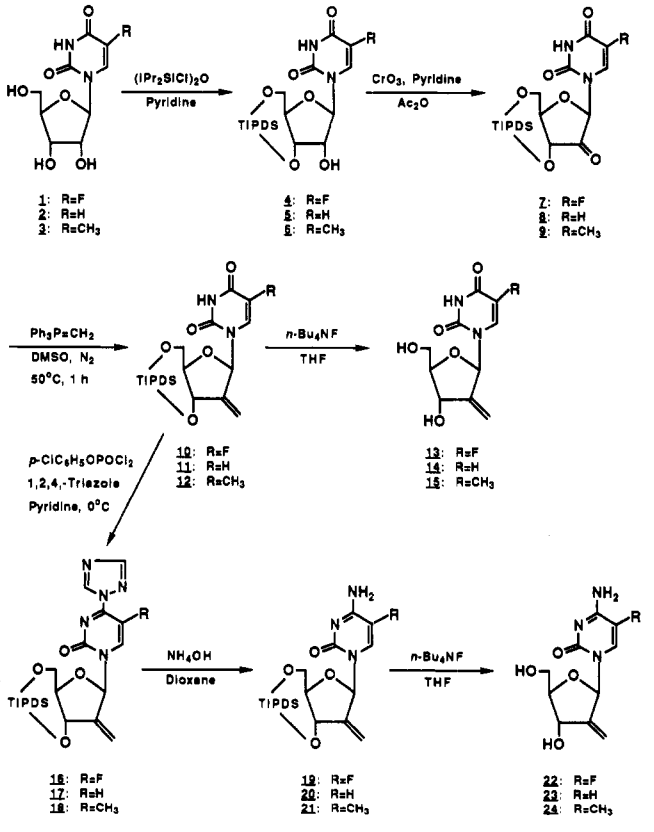
Various 2′- and 3′-methylidene-substituted nucleoside analogues have been synthesized and evaluated as potential anticancer and/or antiviral agents. Among these compounds, 2′-deoxy-2′-methylidene-5-fluorocytidine (**22**) and 2′-deoxy-2′-methylidenecytidine (**23**) not only demonstrated potent anticancer activity in culture against murine L1210 and P388 leukemias, Sarcoma 180, and human CCRF-CEM lymphoblastic leukemia, producing ED_{50} values of 1.2 and 0.3 μM, 0.6 and 0.4 μM, 1.5 and 1.5 μM, and 0.05 and 0.03 μM, respectively, but also were active in mice against murine L1210 leukemia. Of all the tested drug dosage levels (25, 50, and 75 mg/kg, respectively) compound **23** had no toxic deaths and compound **22** yielded only one toxic death at the highest dosage level. On the contrary, in the same study, 1-β-D-arabinofuranosylcytosine (ara-C) resulted in 2/5, 5/5, and 5/5 toxic deaths, respectively. Both compounds **22** and **23** have shown better anticancer activity than ara-C, yielding higher $T/C \times 100$ values and some long-term survivors (>60 days). In addition, compounds **22** and **23** were found to have, respectively, approximately 130 and 40 times lower binding affinity for cytidine/deoxycytidine deaminase derived from human KB cells compared to ara-C, suggesting that the two 2′-methylidene-substituted analogues may be more resistant to deamination. Cytoplasmic deoxycytidine kinase (dCK) was required for compounds **22** and **23** action. Furthermore, compounds **14**, **22**, **23**, and **24** also have antiherpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) activity in cell culture. In addition, the crystal structure of 2′-deoxy-2′-methylidenecytidine hydrochloride (**23**·HCl) was determined by X-ray crystallography.

Various natural and synthetic cytosine nucleosides such as 1-β-D-arabinofuranosylcytosine (ara-C) and 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) have been shown to possess potent anticancer[2] and antiviral[3] activity, respectively. However, many of these biologically active cytosine nucleosides either have a therapeutic index that is too low for human use or their anticancer and/or antiviral efficacies are diminished by their susceptibility to rapid deamination[4-6] to inactive uracil nucleoside analogues. Hence, many structural modifications have been made in an attempt to circumvent these problems.[7-16] For example, replacing the 3′-hydroxyl group in the sugar moiety of 2′-deoxycytidine with an amino group, producing 3′-amino-2′,3′-dideoxycytidine,[13] not only has potent anticancer activity both in vitro[13,17] and in tumor-bearing mice[13,18] but also was found to be resistant to deamination by cytidine/deoxycytidine deaminase isolated from human KB cells.[13,17]

On the basis of these findings, various 2′- and 3′-methylidene-substituted nucleoside analogues have been synthesized as potential anticancer and/or antiviral agents. Our preliminary results have shown that some of the compounds, such as 2′-deoxy-2′-methylidene-5-fluorocytidine (**22**) and 2′-deoxy-2′-methylidenecytidine (**23**), not only demonstrated potent anticancer activity in culture and in tumor-bearing mice but also were resistant to deamination by cytidine/deoxycytidine deaminase derived from human KB cells. In addition 2′-deoxy-2′-methylideneuridine (**14**) has shown antiviral activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro; however, 2′-deoxy-2′-methylidene-5-methyluridine (**15**) was devoid of any antiviral activity.

In this report, the synthesis and anticancer and antiviral activities as well as some of the biochemical studies of these compounds and the X-ray crystallographic analysis of

Scheme I[a]



[a] TIPDS = 1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl.

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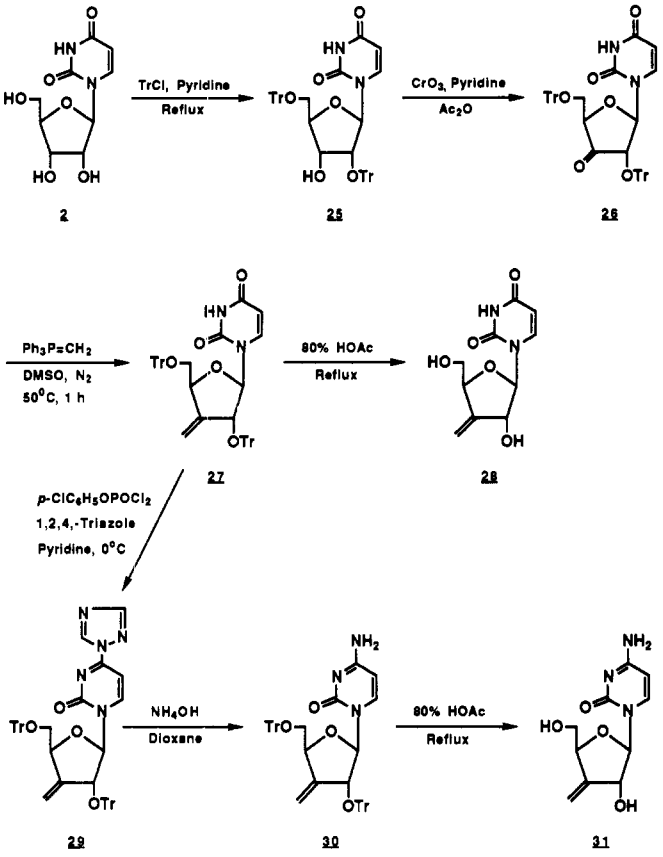
Scheme II



2′-deoxy-2′-methylidenecytidine hydrochloride (**23**·HCl) are described.

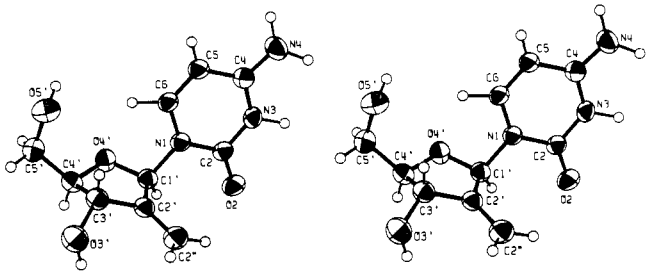


Figure 1. Stereoscopic view of the 2′-deoxy-2′-methylidenecytidine hydrochloride molecule.

Chemistry

2′-Deoxy-2′-methylidene-5-fluorouridine (**13**), 2′-deoxy-2′-methylideneuridine (**14**), 2′-deoxy-2′-methylidene-5-methyluridine (**15**), 2′-deoxy-2′-methylidene-5-fluorocytidine (**22**), 2′-deoxy-2′-methylidenecytidine (**23**), and 2′-deoxy-2′-methylidene-5-methylcytidine (**24**) were synthesized as depicted in Scheme I. Treatment[19] of 5-fluorouridine (**1**), uridine (**2**), and 5-methyluridine (**3**) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine gave the corresponding 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivatives **4**, **5**, and **6**. Oxidation[20] of compounds **4**–**6** with chromium trioxide/pyridine/acetic anhydride complex (1:2:1, molar ratio) in methylene chloride produced the respective 2′-keto nucleosides **7**, **8**, and **9**, which were then converted to the 2′-methylidene analogues **10**, **11**, and **12** by reaction[21] with methylenetriphenylphosphorane in anhydrous Me_2SO at 50 °C under nitrogen. Deprotection[19] of compounds **10**–**12** with tetra-*n*-butylammonium fluoride in THF gave the desired products **13**, **14**, and **15**, respectively. Treatment[12,22] of

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compounds 10–12 with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature yielded the 4-triazolylpyrimidinone derivatives 16–18. Subsequent treatment[12] of 16–18 with a mixture of ammonium hydroxide/dioxane (1:3) produced the corresponding cytidine derivatives 19–21, which were then deblocked by reaction[19] with tetra-*n*-butylammonium fluoride to afford the cytidine analogues 22, 23, and 24. Compound 23 was also independently synthesized by Takenuki et al.[16] using a different methodology.

The syntheses of 3'-deoxy-3'-methylideneuridine (28) and 3'-deoxy-3'-methylidenecytidine (31) are shown in Scheme II. The intermediate, 2',5'-ditrityluridine (25), was prepared from uridine (2) by the procedure of Cook et al.[23] with minor modification. Oxidation[20] of 25 with CrO_3/pyridine/Ac_2O yielded the 3'-carbonyl compound 26, which was then converted to the corresponding 3'-methylidene derivative by Wittig reaction[21] according to the previously described methodology. Detritylation of 27 by refluxing with 80% acetic acid afforded the desired 3'-deoxy-3'-methylideneuridine (28). Treatment[12,22] of compound 27 with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in anhydrous pyridine at room temperature gave the 4-triazolylpyrimidinone derivative 29. Subsequent treatment[12] of 29 with aqueous ammonia in dioxane yielded the protected 3'-deoxy-3'-methylidenecytidine derivative 30, which was then deblocked by reflux with 80% acetic acid to afford the desired compound 31.

X-ray Crystal Structure Analysis of 2'-Deoxy-2'-methylidenecytidine Hydrochloride (23·HCl). In order to determine the conformation of the molecule, and in particular the hitherto unknown effect of the exocyclic double bond in the sugar moiety, we carried out an X-ray analysis. A stereoscopic view of the molecule is shown in Figure 1. As expected, the sp^2 hybridization of C2' causes a flattening of the furanose ring. The maximum degree of pucker[24] $\tau_m = 31.7°$, not very much lower than the usual 37°. The phase angle of pseudorotation $P = 42.3°$ is somewhat outside normal limits and indicates a C4' exo/C3' endo ($_4T^3$) pucker. The CH_2OH side chain adopts the common gauche$^+$ conformation and the conformation about the glycosidic bond (C6–N1–C1'–O4') is anti ($\chi_{CN} = 31.2°$).

The esds of bond distances and angles are 0.003–0.004 Å and 0.2–0.3°, respectively. In the sugar ring, the sp^2 hybridized C2' causes C1'–C2' (1.505 Å) and C2'–C3' (1.494 Å) to be about 0.03 Å shorter than normal, while C1'–C2'–C3' (107.1 Å) is about 5° larger than normal. The pyrimidine ring is protonated at N3 and its geometry is in very good agreement with that of other such rings.[25] The relatively short C4–N4 bond (1.311 Å) indicates a substantial contribution of a resonance form in which the N3–C4 bond moved to C4–N4, thus disrupting the conjugation of the double-bond system. As a result, the C2–O2 bond is shorter (1.218 Å), i.e., has more double-bond character, than in neutral cytosine residues.

Table I. Comparison of the ED_{50} Values of Several Pyrimidine 2'- and 3'-Methylidene-Substituted Deoxynucleoside Analogues with 1-β-D-Arabinofuranosylcytosine (ara-C) on the Replication of L1210, P388, S-180, CCRF-CEM, and CCRF-CEM (dCK$^-$) Cells in Vitro

	ED_{50}[a] values in μM against cell lines				
compd	L1210	P388	S-180	CCRF-CEM	CCRF-CEM (dCK$^-$)[b]
13	22	>100	6.5	30	
14	>100	>100	>100	>100	
15	>100	>100	>100	10	
22	1.2	0.6	1.5	0.05	330
23	0.3	0.4	1.5	0.03	77
24	>100	>100	>100	22	
28	>100	>100	>100	>100	
31	>100	>100	>100	>100	
ara-C	0.05	0.02	0.2	0.006	120

[a] The ED_{50} values were estimated from dose–response curves compiled from at least two independent experiments and represents the drug concentration (μM) required to inhibit replication of the respective L1210, P388, S-180, and CCRF-CEM cell lines by 50% after 72 h incubation. [b] CCRF-CEM (dCK$^-$) is a variant of the CCRF-CEM line and is deficient in cytoplasmic deoxycytidine kinase. This line was kindly provided by Dr. A. Fridland of St. Jude Children's Research Hospital.

All available protons participate in N–H···Cl$^-$ and O–H···Cl$^-$ hydrogen bonds. In addition, there are short intramolecular contacts between H6 and O4' (2.16 Å) and between H6 and O5' (2.62 Å). The latter is quite common and is known to stabilize the gauche$^+$ conformation of the side chain.[26] On the other hand, a C6–H···O4' hydrogen bond is quite unusual; it was first observed in the structure of the antiviral nucleoside 5-(hydroxymethyl)-2'-deoxyuridine.[27]

Biological Evaluation

Anticancer Activity in Vitro. Various pyrimidine 2'- and 3'-methylidene-substituted deoxynucleoside analogues have been evaluated against murine L1210 and P388 leukemias, Sarcoma 180 (S-180), and human CCRF-CEM lymphoblastic leukemia. The activity is expressed as the concentration (μM) required to inhibit cell replication by 50% (ED_{50}) of each of the given cell lines. The results are listed in Table I. Although not as active as 1-β-D-arabinofuranosylcytosine (ara-C), the 2'-methylidenecytidine derivatives, 2'-deoxy-2'-methylidene-5-fluorocytidine (22) and 2'-deoxy-2'-methylidenecytidine (23), produced the greatest activity among the synthesized compounds, with respective ED_{50} values of 1.2 and 0.3 μM against L1210, 0.6 and 0.4 μM against P388, 1.5 and 1.5 μM against S-180, and 0.05 and 0.03 μM against CCRF-CEM. However, Takenuki et al.[16] reported 2'-deoxy-2'-methylidenecytidine (23) to have an ED_{50} of 0.40 μM for inhibition of L1210 cells in vitro. Whereas the ED_{50} for compound 23 is similar in both systems, ara-C is less inhibitory in their system that may be due to partial deamination by their strain of L1210 cells. With the exception of the P388 cancer cell line, 2'-deoxy-2'-methylidene-5-fluorouridine (13) was significantly active with ED_{50} values

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Table II. Effects of 2′-Deoxy-2′-methylidene-5-fluorocytidine (**22**), 2′-Deoxy-2′-methylidenecytidine (**23**), and 1-β-D-Arabinofuranosylcytosine (ara-C) on the Survival of CD_2F_1 Mice Bearing the L1210 Leukemia

compd	dosage,[a] mg/kg	toxic deaths[b]	av days survival[c]	$T/C \times 100$[d]	long-term survivors[e]
control			7.6		
22	25	0/5	24.0	316	2/5
	50	0/5	21.2	279	0/5
	75	1/5	18.5	243	1/5
23	25	0/5	21.6	284	0/5
	50	0/5	21.0	276	0/5
	75	0/5	20.8	273	1/5
ara-C	25	2/5	19.7	258	0/5
	50	5/5	9.6	126	0/5
	75	5/5	8.4	111	0/5

[a] Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, twice daily for 6 consecutive days. [b] Deaths due to toxicity occurring at a maximum body weight decrease of 10 to 22% from the average post injection body weight. [c] Average survival time includes only those mice that died prior to day 60. [d] $T/C \times 100$ represents the ratio of the survival time of treated to control animals × 100. The average survival time of the untreated tumor-bearing control animals was 7.6 days. [e] Long-term survivors are the number of mice that survived for >60 days relative to the number of treated mice.

of 22 μM against L1210, 6.5 μM against S-180, and 30 μM against CCRF-CEM. However, 2′-deoxy-2′-methylidene-5-methyluridine (**15**) and 2′-deoxy-2′-methylidene-5-methylcytidine (**24**) demonstrated activity only against CCRF-CEM with ED_{50} values of 10 and 22 μM, respectively. Conversely, 2′-deoxy-2′-methylideneuridine (**14**), 3′-deoxy-3′-methylideneuridine (**28**), and 3′-deoxy-3′-methylidenecytidine (**31**) showed no activity at concentrations up to 100 μM.

Compounds **22** and **23** were also examined against CCRF-CEM deoxycytidine kinase deficient variant [CCRF-CEM (dCK^-)] and ED_{50} values were much higher than those against CCRF-CEM. These results suggest that cytoplasmic deoxycytidine kinase played a critical role for their action and could be responsible for their phosphorylation to monophosphate nucleosides.

It is noteworthy to mention that compounds 2′-deoxy-2′-methylidene-5-fluorocytidine (**22**) and 2′-deoxy-2′-methylidenecytidine (**23**) demonstrated much greater activity than their 2′-methylideneuridine counterparts, 2′-deoxy-2′-methylidene-5-fluorouridine (**13**) and 2′-deoxy-2′-methylideneuridine (**14**). This illustrates that the activity does not require deamination of compounds **22** and **23** to the uridine form.

Anticancer Activity in Vivo. The most active compounds of the 2′-methylidenecytidine series in vitro, 2′-deoxy-2′-methylidene-5-fluorocytidine (**22**) and 2′-deoxy-2′-methylidenecytidine (**23**), along with ara-C for comparison, were screened against L1210 leukemia bearing CD_2F_1 female mice. The compounds were administered by intraperitoneal (ip) injection in groups of five mice with respective dosage levels of 25, 50, and 75 mg/kg twice a day, for 6 consecutive days. The results of this investigation are summarized in Table II.

The control mice died on an average of 7.6 days after tumor implantation and experienced weight gain up to 11% above body weight recorded before tumor implantation. The mice that received 50 or 75 mg/kg of ara-C died after an average of 9.6 ($T/C \times 100 = 126$) and 8.4 ($T/C \times 100 = 111$) days, respectively. However, for these two groups, the dramatic loss in body weight post drug injection (an average decrease of 20% from the original body weight) indicates that their deaths were attributed to drug toxicity rather than the leukemia.

At 25 mg/kg of ara-C, there was an increase in the average survival time to 19.7 days, yielding a $T/C \times 100$ value of 258. This dosage was less toxic. An average 10% decrease from the original body weight was noted in the first two mice that died on days 10 and 14. The three remaining mice died 8 to 11 days later on days 18, 20, and 21, respectively, and had an average 7% increase in body weight due to the growth of the tumor. Because ara-C at this dosage did not produce any long-term survivor(s) among the remaining mice, the dosage level below 25 mg/kg of ara-C was not tested.

2′-Deoxy-2′-methylidene-5-fluorocytidine (**22**) showed less toxicity than ara-C and produced three long-term (>60 days) survivors. The group receiving 25 mg/kg of compound **22** gave the highest average life span, 24 days, yielding a $T/C \times 100$ value of 316 and had 2 long-term survivors. Initially, on day 7, toxicity was demonstrated by an average decrease of 8% from the original body weight, but the weight returned to normal within 2 days. Mice receiving a dosage of 50 mg/kg had an average life span of 21.2 days, producing a $T/C \times 100$ value of 279, but also showed an increase in toxicity since an average decrease of 19% from the original body weight on day 8 was observed. However, these mice were able to rebound to their original weight by day 12. At the 75 mg/kg dosage level, the average survival time for the mice was 18.5 days ($T/C \times 100 = 243$) and there was one long-term survivor. In addition, this drug dosage demonstrated rather high toxicity. One mouse died on day 6 before the final injection was administered. The remaining mice experienced the greatest average decrease of 27% from the original body weight on day 10 and took the longest time, 8 days later, to rebound to their original weight.

2′-Deoxy-2′-methylidenecytidine (**23**) showed negligible variation in the results in all three dosage levels. Although the 75 mg/kg dosage did produce one long-term survivor, all dosage levels gave similar average survival times of 21.6, 21.0, and 20.8 days, respectively, yielding corresponding $T/C \times 100$ values of 284, 276, and 273. There was a small average decrease of 5% from the original body weight noted in all groups, indicating that toxicity was not a factor with this compound.

Although ara-C was found to be more active in tissue culture against L1210 leukemia than both compounds **22** and **23**, the in vivo tests against the same cell line in tumor-bearing mice did not give the corresponding results. Ara-C, on the contrary, was found to be much more toxic than both compounds **22** and **23** and did not produce any long-term survivors in any of the tested dosage levels. At comparable dosages, compound **23** demonstrated minimal toxicity, compound **22** was somewhat more toxic, and ara-C showed the greatest toxicity. Of all tested drug dosage levels, compound **23** had no toxic deaths and compound **22** produced only one toxic death at the highest dosage level (75 mg/kg). Conversely, ara-C resulted in 2/5, 5/5, and 5/5 toxic deaths for the animals tested at the respective 25, 50, and 75 mg/kg dosage levels. In addition, compounds **22** and **23** demonstrated better anticancer activity than ara-C, yielding higher $T/C \times 100$ values and some long-term survivors. Since both compounds **22** and **23** have a much better therapeutic index than ara-C in the in vivo studies at all dosages and also produced several long-term survivors, whereas ara-C did not, further biological and biochemical investigations are merited.

Antiviral Activity in Vitro. Several compounds were examined against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) growth in Hela cells. The concentration required to inhibit 90% of virus yield during a 24-h growth

Table III. Effect of Pyrimidine 2'- and 3'-Methylidene-Substituted Deoxynucleoside Analogues against Herpes Simplex Virus Type 1 (HSV-1) and Type 2 (HSV-2) Growth

compd	ID_{90}[a] (μM) HSV-1	HSV-2
13	>250	>250
14	50[b]	50[b]
22	50	5
23	26	5
24	32	125
31	>250	>250

[a] ID_{90} represents the drug concentration (μM) required to inhibit 90% of virus yield during a 24-h growth period. [b] 80% Inhibition of yield was achieved with 50 μM of compound 14. There was no more inhibition up to 250 μM.

Table IV. Inhibitory Constants (K_i) of Selected 2'- and 3'-Methylidene-Substituted Deoxycytidine Analogues by Cytidine/Deoxycytidine Deaminase[a]

compd	K_i (mM)	r^2
22	25.1 ± 2.2	0.99887
23	7.69 ± 1.2	0.99634
31	1.60 ± 0.13	0.99779
ara-C	0.19 ± 0.015	0.99869

[a] The enzyme assay was performed by using radiolabeled 2'-deoxycytidine at fixed concentrations against varying concentrations of the particular analogue. The data was fit to the rate equation for competitive inhibition using a nonlinear least-squares computer program. K_i values are shown with the calculated standard deviation. In the absence of analogue, the K_m for 2'-deoxycytidine was 32.5 ± 3.6 μM (r^2 = 0.99855).

period was designated as ID_{90}. The assay procedures were the same as published previously.[28] The results are shown in Table III. Some of the compounds have potent antiviral activity, but none illustrated select antiviral activity against HSV-1 or HSV-2 at the concentration nontoxic to cells. Deoxycytidine analogues are more potent inhibitors than deoxyuridine analogues. It is likely that the observed antiviral activity was mediated through host nucleoside kinases and not through virus specified thymidine kinase.

Biochemical Studies. Since it is known that drug inactivation by deamination is responsible for the short biological half-life of ara-C,[4,29,30] it was necessary to determine the susceptibility to enzymatic deamination of biologically active analogues containing the 4-amino group. This is especially important for compounds **22** and **23** because the corresponding uridine derivatives, **13** and **14**, respectively, are much less toxic to malignant cells (Table I).

As depicted in Table IV, an inhibition constant (K_i) was determined for each analogue in the reaction catalyzed by cytidine/deoxycytidine (Cyd/dCyd) deaminase that was isolated from human KB cells. As can be seen, ara-C had the lowest K_i value, implying that it is a good substrate for this enzyme consistent with published reports.[4,29,30] The 3'-deoxy-3'-methylidenecytidine (**31**) is also expected to be deaminated by this enzyme. However, this is academic since this compound showed no toxicity against cultured cells (Table I), probably because it is an analogue of cytidine and therefore not a substrate for dCyd kinase, the rate-limiting enzyme required for biological activity of 2'-deoxycytidine analogues.

2'-Deoxy-2'-methylidene-5-fluorocytidine (**22**) and 2'-deoxy-2'-methylidenecytidine (**23**) demonstrated the lowest binding affinity for the deaminase as is evident from the high K_i values, suggesting that compounds **22** and **23** are 132 and 40 times, respectively, more resistant to deamination than ara-C, especially compound **22**, which is probably not a substrate for this enzyme under physiological conditions. Moreover, the potent antiproliferative effect induced by these agents against cultured cells suggests that the nucleotide derivative is formed by enzymes within cells, the first of which is believed to be dCyd kinase.

As reported previously,[13,17,18] the cytotoxic 3'-amino analogue of 2'-deoxycytidine is also phosphorylated by dCyd kinase and is not a substrate for Cyd/dCyd deaminase. This agent, which is still under investigation, specifically inhibits DNA biosynthesis,[13] possibly by termination of the DNA chain during replication.[31] However, chain termination is not the expected mode of action for the 2'-methylidene analogues **22** and **23** of the present study in view of the unmodified 3'-hydroxyl moiety. This together with the observed low binding affinity for Cyd/dCyd deaminase suggests that these compounds warrant further evaluation as anticancer agents.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 (90 MHz) NMR spectrometer or a Bruker WM-500 (500 MHz) spectrometer (for the final products, **13–15**, **22–24**, **28**, and **31**) with Me_4Si as the internal reference. Chemical ionization (CI-MS) mass spectra were determined with a Kratos MS80 RFA high resolution instrument. The UV spectra were recorded on a Beckman-25 spectrophotometer. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value.

3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-5-fluorouridine (4). A mixture of 5-fluorouridine (1, 2.95 g, 11.2 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.60 g, 11.4 mmol) in pyridine (40 mL) was stirred at room temperature for 2 days. The solvent was removed in vacuo at ~30 °C. The resulting residue was dissolved in methylene chloride (50 mL) and washed with water (3 × 30 mL). The methylene chloride solution was dried with anhydrous $MgSO_4$ and filtered. The filtrate was then reduced to a small volume in vacuo and chromatographed on a silica gel column ($CHCl_3$/MeOH, 20:1, v/v). The fractions containing the desired product (R_f 0.21) were pooled together and the solvent was evaporated in vacuo again to afford 5.1 g (90%) of a foam: NMR ($CDCl_3$) δ 1.05–1.15 [m, 28 H, $CH(CH_3)_2$], 4.44–4.75 (m, 6 H, 2'-H, 3'-H, 4'-H, and 5'-H; and 2'-OH, D_2O exchangeable), 5.50 (d, 1 H, 1'-H), 7.35 (d, 1 H, 6-H). Anal. ($C_{21}H_{37}N_2O_7Si_2F$) C, H, N.

3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)uridine (5):[19] yield 10.6 g (76%), as a foam; TLC, R_f 0.76 (CH_2Cl_2/MeOH, 10:1, v/v); NMR ($CDCl_3$) δ 1.10–1.20 [m, 28 H, $CH(CH_3)_2$], 4.40–4.70 (m, 6 H, 2'-H, 3'-H, 4'-H, 5'-H; and 2'-OH, D_2O ex-

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changeable), 5.95–6.05 (m, 2 H, 5-H and 1′-H), 8.05 (d, 1 H, 6-H), 10.25 (s, 1 H, 3-NH, D_2O exchangeable).

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (6): yield 1.6 g (69%), as a foam; TLC, R_f 0.77 (CH_2Cl_2/MeOH, 10:1, v/v); NMR ($CDCl_3$) δ 1.10–1.20 [m, 28 H, $CH(CH_3)_2$], 2.00 (s, 3 H, 5-CH_3), 3.52 (s, 1 H, 2′-OH, D_2O exchangeable), 4.10–4.30 (m, 5 H, 2′-H, 3′-H, 4′-H, and 5′-H), 5.72 (d, 1 H, 1′-H), 7.42 (d, 1 H, 6-H), 9.35 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{22}H_{40}N_2O_7Si_2$) C, N, H calcd 5.60, found 5.14.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-keto-5-fluorouridine (7). To a stirred suspension of CrO_3 (0.9 g), pyridine (1.5 mL), and acetic anhydride (0.9 mL) in CH_2Cl_2 (20 mL) was added compound 4 (1.6 g, 3.2 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for an additional 30 min. The dark brown solution was poured into 130 mL of ethyl acetate, and the resulting mixture was filtered through a 2-cm layer of silica gel in a 4-cm diameter sinter glass filter. The precipitated solids and silica gel were washed with ethyl acetate (100 mL) and the combined filtrates were evaporated in vacuo (<25 °C) to dryness. The residue was coevaporated with toluene (50 mL), followed by chloroform (50 mL), to yield a foam (1.50 g, 93%), which was used directly for the next preparation without further purification: TLC, R_f 0.68 (CH_2Cl_2/EtOAc, 1:1, v/v); UV (MeOH) λ_{max} 268 nm (ε 8266), λ_{min} 235 nm; UV (0.01 N NaOH) λ_{max} 266 nm (ε 9680), λ_{min} 246 nm; UV (0.01 N HCl) λ_{max} 266 nm (ε 9011), λ_{min} 232 nm; NMR ($CDCl_3$) δ 1.05–1.25 [m, 28 H, $CH(CH_3)_2$], 4.05–4.25 (m, 4 H, 3′-H, 4′-H, and 5′-H), 5.15 (s, 1 H, 1′-H), 7.25 (d, 1 H, 6-H).

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-ketouridine (8):[20] yield 2.7 g (82%), as a foam, which was used directly for the next preparation without further purification; TLC, R_f 0.61 (CH_2Cl_2/EtOAc, 1:1, v/v); UV (MeOH) λ_{max} 264 nm (ε 7143), λ_{min} 232 nm; UV (0.01 N HCl) λ_{max} 266 nm (ε 6275), λ_{min} 234 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ε 8411), λ_{min} 238 nm; NMR ($CDCl_3$) δ 0.95–1.15 [m, 28 H, $CH(CH_3)_2$], 4.02–4.15 (m, 3 H, 4′-H and 5′-H), 4.95–5.05 (m, 2 H, 3′-H and 1′-H), 5.70 (d, 1 H, 5-H), 7.18 (d, 1 H, 6-H), 9.00 (s, 1 H, 3-NH, D_2O exchangeable).

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-keto-5-methyluridine (9): yield 1.6 g (69%), as a foam, which was used directly for the next preparation without further purification; TLC, R_f 0.64 (CH_2Cl_2/EtOAc, 1:1, v/v); UV (MeOH) λ_{max} 261 nm (ε 7566), λ_{min} 228 nm; UV (0.01 N HCl) λ_{max} 264 nm (ε 6740), λ_{min} 233 nm; UV (0.01 N NaOH) λ_{max} 267 nm (ε 8116), λ_{min} 238 nm; NMR ($CDCl_3$) δ 1.00–1.21 [m, 28 H, $CH(CH_3)_2$], 2.10 (s, 3 H, 5-CH_3), 4.02–4.22 (m, 4 H, 3′-H, 4′-H, and 5′-H), 5.10 (s, 1 H, 1′-H), 7.32 (d, 1 H, 6-H).

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-methylidene-5-fluorouridine (10). A suspension of NaH (0.3 g, 80% dispersion in mineral oil, 10 mmol) in 12.5 mL of Me_2SO was heated at 65 °C under nitrogen until all the sodium hydride had dissolved. The solution was cooled to room temperature and methyltriphenylphosphonium bromide (4.0 g, 11 mmol) was added with stirring. After 45 min, compound 7 (1.4 g, 2.8 mmol) was added and the mixture was stirred at 50 °C for 1 h and then evaporated in vacuo to dryness. To the residue was added 100 mL of water, and the pH value of the resulting mixture was adjusted to pH 7 with HOAc. The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined CH_2Cl_2 extract was dried with anhydrous $MgSO_4$ and filtered. The filtrate was then reduced to a small volume in vacuo and chromatographed on a silica gel column (CH_2Cl_2/EtOAc, 1:1, v/v, R_f 0.82) to afford 0.71 g (51%) of a white foam: UV (MeOH) λ_{max} 268 nm (ε 9706), λ_{min} 236 nm; UV (0.01 N NaOH) λ_{max} 267 nm (ε 8936), λ_{min} 239 nm; NMR ($CDCl_3$) δ 0.90–1.10 [m, 28 H, $CH(CH_3)_2$], 3.80 (m, 1 H, 4′-H), 4.22 (m, 2 H, 5′-H), 5.05 (d, 1 H, 3′-H), 5.32–5.65 (d, 2 H, methylidene), 6.62 (s, 1 H, 1′-H), 7.51 (d, 1 H, 6-H). Anal. ($C_{22}H_{37}FN_2O_6Si_2$) C, H, N.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-methylideneuridine (11): yield 0.90 g (34%) as a white foam; TLC, R_f 0.64 (CH_2Cl_2/EtOAc, 1:1, v/v); UV (MeOH) λ_{max} 258 nm (ε 9154), λ_{min} 228 nm; UV (0.01 N HCl) λ_{max} 264 nm (ε 12364), λ_{min} 238 nm; UV (0.01 N NaOH) λ_{max} 264 nm (ε 9417), λ_{min} 236 nm; NMR ($CDCl_3$) δ 0.80–1.00 [m, 28 H, $CH(CH_3)_2$], 3.60 (m, 1 H, 4′-H), 4.02 (m, 2 H, 5′-H), 4.72 (d, 1 H, 3′-H), 5.40 (m, 2 H, methylidene), 5.62 (d, 1 H, 5-H), 6.42 (s, 1 H, 1′-H), 7.33 (d, 1 H, 6 H), 9.68 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{22}H_{38}N_2O_6Si_2$) C, H, N.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-methylidene-5-methyluridine (12): yield 0.9 g (40%) as a white foam; TLC, R_f 0.69 (CH_2Cl_2/EtOAc, 1:1, v/v); UV (MeOH) λ_{max} 266 nm (ε 8258), λ_{min} 236 nm; UV (0.01 N HCl) λ_{max} 272 nm (ε 9923), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ε 9748), λ_{min} 242 nm; NMR ($CDCl_3$) δ 1.05–1.20 [m, 28 H, $CH(CH_3)_2$], 2.10 (s, 3 H, 5-CH_3), 3.65 (m, 1 H, 4′-H), 4.02 (m, 2 H, 5′-H), 4.80 (d, 1 H, 3′-H), 5.40 (m, 2 H, methylidene), 6.50 (s, 1 H, 1′-H), 7.05 (d, 1 H, 6-H), 9.20 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{23}H_{40}N_2O_6Si_2$) C, H, N.

2′-Deoxy-2′-methylidene-5-fluorouridine (13). To a stirred solution of compound 10 (0.65 g, 1.30 mmol) in 10 mL of THF was added 2 mL of 1 M n-$Bu_4N^+F^-$/THF at ambient temperature. The resultant mixture was stirred for 1 h. When TLC showed the reaction was complete, the solution was evaporated in vacuo to dryness and the oily residue was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was evaporated in vacuo to dryness, and the residue was chromatographed on a silica gel column (CH_2Cl_2/MeOH, 6:1, v/v). The product was obtained as almost colorless needles (0.22 g, 65%): mp 170–171 °C; TLC, R_f 0.50 (CH_2Cl_2/MeOH, 6:1, v/v); UV (MeOH) λ_{max} 270 nm (ε 7558), λ_{min} 236 nm; UV (0.01 N HCl) λ_{max} 270 nm (ε 8967), λ_{min} 236 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ε 7840), λ_{min} 250 nm; NMR (Me_2SO-d_6) δ 3.56–3.70 (m, 3 H, 4′-H and 5′-H), 4.50 (m, 1 H, 3′-H), 5.00 (t, 1 H, 5′-OH, D_2O exchangeable), 5.30 [t (overlap dd), 1 H, methylidene-H_A, J = 2.0 Hz], 5.39 [t (overlap dd), 1 H, methylidene-H_B, J = 2.0 Hz], 5.63 (d, 1 H, 3′-OH, D_2O exchangeable), 6.42 [t (overlap dd), 1 H, 1′-H, J = 1.5 Hz), 7.94 (d, 1 H, 6-H, J = 7.0 Hz), 11.9 (s, 1 H, 3-NH, D_2O exchangeable); MS m/z 259 (M^+ + 1), 131 (5-fluorouracil + 1), 129 [$C_6H_9O_3$ (sugar residue)]. Anal. ($C_{10}H_{11}FN_2O_5$) C, H, N.

2′-Deoxy-2′-methylideneuridine (14): yield 0.95 g (88%) as colorless needles; mp 164–165 °C; TLC, R_f 0.56 (EtOAc/EtOH, 6:1, v/v); UV (MeOH) λ_{max} 263 nm (ε 10 570), λ_{min} 232 nm; UV (0.01 N HCl) λ_{max} 270 nm (ε 9737), λ_{min} 232 nm; UV (0.01 N NaOH) λ_{max} 262 nm (ε 10 249), λ_{min} 233 nm; NMR (Me_2SO-d_6) δ 3.56 (m, 2 H, 5′-H), 3.67 (m, 1 H, 4′-H), 4.44 (m, 1 H, 3′-H), 4.93 (t, 1 H, 5′-OH, D_2O exchangeable), 5.21 [t (overlap dd), 1 H, methylidene-H_A, J = 2.1 Hz], 5.38 [t (overlap dd), 1 H, methylidene-H_B, J = 2.1 Hz], 5.62 (d, 1 H, 5-H, J = 8.0 Hz), 5.65 (d, 1 H, 3′-OH, D_2O exchangeable), 6.42 (d, 1 H, 1′-H, J = 1.6 Hz), 7.50 (d, 1 H, 6-H, J = 8.0 Hz), 11.9 (s, 1 H, 3-NH, D_2O exchangeable); MS m/z 241 (M^+ + 1), 129 [$C_6H_9O_3$ (sugar residue)], 113 (uracil + 1). Anal. ($C_{10}H_{12}N_2O_5$) C, H, N.

2′-Deoxy-2′-methylidene-5-methyluridine (15): yield 0.58 g (91%), as colorless needles; mp 155–157 °C; TLC, R_f 0.53 (EtOAc/EtOH, 6:1, v/v); UV (MeOH) λ_{max} 268 nm (ε 8531), λ_{min} 236 nm; UV (0.01 N HCl) λ_{max} 268 nm (ε 10 396), λ_{min} 237 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ε 10 960), λ_{min} 237 nm; NMR (Me_2SO-d_6) δ 1.80 (s, 3 H, 5-CH_3), 3.52–3.70 (m, 3 H, 4′-H and 5′-H), 4.44 (m, 1 H, 3′-H), 4.93 (t, 1 H, 5′-OH, D_2O exchangeable), 5.21 (dd, 1 H, methylidene-H_A, J = 2.0 Hz, 1.6 Hz), 5.37 (dd, 1 H, methylidene-H_B, J = 2.0 Hz, 1.7 Hz), 5.65 (d, 1 H, 3′-OH, D_2O exchangable), 6.48 (d, 1 H, 1′-H, J = 1.6 Hz), 7.38 (d, 1 H, 6-H), 11.4 (s, 1 H, 3-NH, D_2O exchangeable); MS m/z 255 (M^+ + 1), 129 [$C_6H_9O_3$ (sugar residue)], 127 (thymine + 1). Anal. ($C_{11}H_{14}N_2O_5$) C, H, N.

1-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-deoxy-2-methylidene-β-D-ribofuranosyl]-4-(1,2,4-triazol-1-yl)-1*H*-pyrimidin-2-one (17). To a cooled solution (0 °C) of compound 11 (1.88 g, 3.90 mmol) and 1,2,4-triazole (3.54 g, 51.3 mmol) in anhydrous pyridine (24 mL) was added dropwise 4-chlorophenyl phosphorodichloridate (2.8 mL, 17.1 mmol) with stirring. After the reaction mixture was stirred at room temperature for 2 days, the solvent was evaporated in vacuo to yield a syrup, which was dissolved in CH_2Cl_2 (50 mL) and washed with water (4 × 25 mL). The CH_2Cl_2 solution was dried ($MgSO_4$) and filtered. The filtrate was concentrated to a small volume in vacuo and purified by silica gel column chromatography (eluting with EtOAc, R_f 0.67) to afford 1.6 g (77%) of product as a white foam. Anal. ($C_{24}H_{39}N_5O_5Si_2$) C, H, N.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-methylidene-5-fluorocytidine (19). 4-Chlorophenyl phosphorodichloridate (1.2 mL, 7.1 mmol) was added dropwise

to a cooled solution of compound **10** (0.8 g, 1.6 mmol) and 1,2,4-triazole (1.45 g, 21 mmol) in 20 mL of pyridine (ice/water bath). The mixture was stirred at room temperature for 2 days and monitored by TLC until the reaction was completed. The mixture was evaporated in vacuo to dryness, and the residue was dissolved in CH_2Cl_2 (30 mL), washed with water (4 × 10 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated in vacuo to give the crude form of **16** (unstable by silica gel chromatography purification), which was dissolved in NH_4OH/dioxane (66 mL, 1:2, v/v). The resulting solution was stirred at room temperature for 4 h and then evaporated in vacuo to dryness. The residue was dissolved in 50 mL of CH_2Cl_2, washed with water (2 × 30 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness in vacuo to give a foam (0.71 g, 89%): R_f 0.30 (CH_2Cl_2/MeOH, 20:1, v/v); NMR ($CDCl_3$) δ 0.85–1.10 [m, 28 H, $CH(CH_3)_2$], 3.55 (m, 1 H, 4'-H), 3.95 (d, 2 H, 5'-H), 4.55 (d, 1 H, 3'-H), 5.25 (d, 1 H, methylidene-H_A), 5.42 (d, 1 H, methylidene-H_B), 6.32 (s, 1 H, 1'-H), 7.38 (d, 1 H, 6-H), 8.10 (s, 2 H, 4-NH_2, D_2O exchangeable).

This product was used for the next preparation without further purification.

3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-deoxy-2'-methylidenecytidine (20). Compound **17** (1.5 g, 2.8 mmol) was dissolved in a solution of NH_4OH (10 mL) and dioxane (30 mL) and stirred overnight at room temperature. The solvent was then evaporated to dryness in vacuo. The residue was dissolved in a small amount of CH_2Cl_2 and purified by silica gel column chromatography (CH_2Cl_2/MeOH, 4:1, v/v, R_f 0.88) to afford 1.3 g (96%) of product as a foam: NMR ($CDCl_3$) δ 1.00–1.10 [m, 28 H, $CH(CH_3)_2$], 3.70 (m, 1 H, 4'-H), 4.10 (d, 2 H, 5'-H), 4.75 (d, 1 H, 3'-H), 5.40 (d, 1 H, methylidene-H_A), 5.55 (d, 1 H, methylidene-H_B), 5.80 (d, 1 H, 5-H), 6.65 (s, 1 H, 1'-H), 7.50 (d, 1 H, 6-H), 8.15 (s, 2 H, 4-NH_2, D_2O exchangeable). Anal. ($C_{22}H_{39}N_3O_6Si_2$) C, H, N.

3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-deoxy-2'-methylidene-5-methylcytidine (21). This compound was synthesized from **12** (1 g, 2 mmol) by the same methodology as described for the synthesis of **19**: yield 0.4 g (40%), as a foam; TLC, R_f 0.57 (CH_2Cl_2/MeOH, 10:1, v/v); NMR ($CDCl_3$) δ 1.00–1.10 [m, 28 H, $CH(CH_3)_2$], 1.80 (s, 3 H, 5-CH_3), 3.62 (m, 1 H, 4'-H), 3.95 (d, 2 H, 5'-H), 4.90 (d, 1 H, 3'-H), 5.25 (d, 1 H, methylidene-H_A), 5.35 (d, 1 H, methylidene-H_B), 6.40 (s, 1 H, 1'-H), 6.90 (s, 1 H, 4-NH_A, D_2O exchangeable), 7.15 (d, 1 H, 6-H), 7.40 (s, 1 H, 4-NH_B, D_2O exchangeable). Anal. ($C_{23}H_{41}N_3O_5Si_2$) C, H, N.

2'-Deoxy-2'-methylidene-5-fluorocytidine (22). To a stirred solution of compound **19** (0.65 g, 1.3 mmol) in THF (15 mL) was added dropwise 6 mL of 1 M *n*-$Bu_4N^+F^-$/THF at ambient temperature. The reaction was completed after 1.5 h and the solvent was evaporated in vacuo to dryness. The residue was dissolved in 30 mL of water and extracted with CH_2Cl_2 (2 × 20 mL). The water layer was evaporated in vacuo and the residue was chromatographed on a silica gel column (R_f 0.29, CH_2Cl_2/MeOH, 8:1 v/v) to afford 0.26 g (78%) of white crystals: mp 163–165 °C; UV (MeOH) λ_{max} 285 nm (ε 6982), λ_{min} 267 nm; UV (0.01 N HCl) λ_{max} 287 nm (ε 9113), λ_{min} 253 nm; UV (0.01 N NaOH) λ_{max} 282 nm (ε 7939), λ_{min} 265 nm; NMR (Me_2SO-d_6) δ 3.60 (m, 2 H, 5'-H), 3.71 (m, 1 H, 4'-H), 4.48 (m, 1 H, 3'-H), 5.04 (t, 1 H, 5'-OH, D_2O exchangeable), 5.19 [t (overlap dd), 1 H, methylidene-H_A, J = 2.1 Hz], 5.32 [t (overlap dd), 1 H, methylidene-H_B, J = 2.1 Hz], 5.31 (t, 1 H, 5'-OH, D_2O exchangeable), 5.60 (d, 1 H, 3'-OH, D_2O exchangeable), 6.45 [t (overlap dd), 1 H, 1'-H, J = 1.6 Hz], 7.57 and 7.81 (two s, 2 H, 4-NH_2, D_2O exchangeable), 7.83 (d, 1 H, 6-H, J = 6.8 Hz); MS m/z 258 (M^+ + 1), 130 (5-fluorocytosine + 1), 129 [$C_6H_9O_3$ (sugar residue)]. Anal. ($C_{10}H_{12}FN_3O_4$) C, H, N.

Compounds **23** and **24** were synthesized by the same methodology as described for the synthesis of compound **22**.

2'-Deoxy-2'-methylidenecytidine (23) and its hydrochloride salt: yield 0.6 g (92%), as a foam; TLC, R_f 0.51 (CH_2Cl_2/MeOH, 2:1, v/v); UV (MeOH) λ_{max} 277 nm (ε 9623), λ_{min} 250 nm; UV (0.01 N HCl) λ_{max} 278 nm (ε 14 541), λ_{min} 246 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ε 10 412), λ_{min} 250 nm; NMR (Me_2SO-d_6) δ 3.58 (m, 2 H, 5'-H), 3.64 (m, 1 H, 4'-H), 4.43 (m, 1 H, 3'-H), 4.92 (t, 1 H, 5'-OH, D_2O exchangeable), 5.13 [t (overlap dd), 1 H, methylidene-H_A, J = 2.0 Hz], 5.29 [t (overlap dd), 1 H, methylidene-H_B, J = 2.0 Hz], 5.61 (d, 1 H, 3'-OH), D_2O exchangeable), 5.71 (d, 1 H, 5-H, J = 7.4 Hz), 6.51 (d, 1 H, 1'-H, J = 1.6 Hz), 7.10 (d, 2 H, 4-NH_2, D_2O exchangeable), 7.47 (d, 1 H, 6-H, J = 7.4 Hz); MS m/z 240 (M^+ + 1), 129 [$C_6H_9O_3$ (sugar residue)], 112 (cytosine + 1).

Compound **23** (150 mg, 0.63 mmol) was converted to its hydrochloride salt by treatment with acetyl chloride (400 mg, 5 mmol) in MeOH (15 mL), followed by evaporation in vacuo to dryness and crystallization from ethanol, to afford 110 mg (64%) of product as white crystals: darkening at 145 °C and completely decomposed over 300 °C (lit.[16] mp > 300 °C); UV (MeOH) λ_{max} 280 nm (ε 9511), λ_{min} 252 nm; UV (0.01 N HCl) λ_{max} 280 nm (ε 14 611), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ε 10 476), λ_{min} 252 nm; NMR (Me_2SO-d_6) δ 3.58–3.67 (m, 3 H, 4'-H and 5'-H), 3.69 (br, 1 H, 5'-OH, D_2O exchangeable), 4.49 (m, 1 H, 3'-H), 5.37 (d, 1 H, methylidene-H_A, J = 1.5 Hz), 5.42 [t (overlap dd), 1 H, methylidene-H_B, J = 1.9 Hz], 5.60 (d, 1 H, 3'-OH, D_2O exchangeable), 6.13 (d, 1 H, 5-H, J = 7.8 Hz), 6.44 (d, 1 H, 1'-H, J = 1.3 Hz), 7.93 (d, 1 H, 6-H, J = 7.8 Hz), 8.73 and 9.81 (two s, 2 H, 4-NH_2, D_2O exchangeable). Anal. ($C_{10}H_{14}ClN_3O_4$) C, H, N.

2'-Deoxy-2'-methylidene-5-methylcytidine (24): yield 0.12 g (60%); mp 199–201 °C; TLC, R_f 0.52 (CH_2Cl_2/MeOH, 2:1, v/v); UV (MeOH) λ_{max} 275 nm (ε 7169), λ_{min} 257 nm; UV (0.01 N HCl) λ_{max} 283 nm (ε 10 716), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 278 nm (ε 8326), λ_{min} 258 nm; NMR (Me_2SO-d_6) δ 1.80 (s, 3 H, 5-CH_3), 3.55 (m, 2 H, 5'-H), 3.65 (m, 1 H, 4'-H), 4.50 (m, 1 H, 3'-H), 4.90 (t, 1 H, 5'-OH, D_2O exchangeable), 5.10 [t (overlap dd), 1 H, methylidene-H_A, J = 2.1 Hz], 5.28 [t (overlap dd), 1 H, methylidene-H_B, J = 2.2 Hz], 5.55 (d, 1 H, 3'-OH, D_2O exchangeable), 6.50 (d, 1 H, 1'-H, J = 1.7 Hz), 6.82 and 7.32 (two s, 2 H, 4-NH_2, D_2O exchangeable), 7.28 (s, 1 H, 6-H); MS m/z 254 (M^+ + 1), 129 [$C_6H_9O_3$ (sugar residue)], 126 (5-methylcytosine + 1). Anal. ($C_{11}H_{15}N_3O_4$) C, H, N.

2',5'-Di-*O*-trityluridine (25). A mixture of uridine (**2**, 15 g, 61.5 mmol) and chlorotriphenylmethane (51.4 g, 183 mmol) in pyridine (150 mL) was stirred at room temperature overnight and then at 100 °C for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (300 mL), washed with water (4 × 100 mL), dried ($MgSO_4$), and evaporated in vacuo to afford a syrup, which was then crystallized from benzene/ether to produce 14 g (31%) of product; mp 205–207 °C (lit.[23] mp 217–220 °C).

2',5'-Di-*O*-trityl-3'-ketouridine (26). A mixture of CH_2Cl_2 (14 mL), CrO_3 (0.6 g), pyridine (1.0 mL), and acetic anhydride (0.6 mL) was stirred for 3 min, after which compound **25** (1.5 g, 2 mmol) was added to the mixture and allowed to stir at room temperature for another 45 min. The dark brown solution was poured into 100 mL of ethyl acetate, and the resulting mixture was filtered through a 2-cm layer of silica gel in a 4-cm diameter sinter glass filter. The precipitated solid and silica gel were washed with ethyl acetate and the combined filtrates were evaporated (<25 °C) to dryness in vacuo. The residue was coevaporated with toluene (50 mL), followed by chloroform (50 mL), to give a foam, which was crystallized from methanol: yield, 1.2 g (82%); mp 138–140 °C (lit.[23] yield, 66%; mp 146–148 °C).

2',5'-Di-*O*-trityl-3'-deoxy-3'-methylideneuridine (27). A suspension of NaH (1.2 g, 80% dispersion in mineral oil, 40 mmol) in Me_2SO (12.5 mL) was heated at 65 °C under nitrogen until all the sodium hydride had dissolved. The solution was cooled to 20 °C and methyltriphenylphosphonium bromide (14.3 g, 40 mmol) was added with stirring. After 45 min, compound **26** (8.0 g, 11 mmol) was added and the mixture was stirred at 50 °C for 1 h and then evaporated in vacuo to dryness. To the residue was added 100 mL of water, and the pH value of the resulting mixture was adjusted to pH 7 with HOAc. The aqueous solution was extracted with CH_2Cl_2 (3 × 150 mL). The combined CH_2Cl_2 extract was dried with anhydrous $MgSO_4$ and filtered. The filtrate was then reduced to a small volume in vacuo and chromatographed on a silica gel column ($CHCl_3$/EtOAc, 10:1, v/v, R_f 0.46) to afford 1.3 g (16.2%) as a white foam: NMR ($CDCl_3$) δ 3.21 (d, 2 H, 5'-H), 4.47 (d, 1 H, 2'-H), 4.80–5.00 (m, 3 H, 5-H and methylidene), 6.10 (d, 1 H, 1'-H), 7.18–7.38 (m, 30 H, Ar H), 7.66 (d, 1 H, 6-H), 8.76 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{48}H_{40}N_2O_5 \cdot H_2O$) C, H, N.

3'-Deoxy-3'-methylideneuridine (28). Compound **27** (1.0 g, 1.38 mmol) was heated in 80% acetic acid at 75–80 °C for 2.5 h.

The cooled reaction mixture was filtered and the filtrate was evaporated in vacuo to dryness. The residue was chromatographed on a silica gel column (R_f 0.25, CH_2Cl_2/MeOH, 10:1, v/v) to afford 0.31 g (94%) of white foam: UV (MeOH) λ_{max} 263 nm (ϵ 6982), λ_{min} 237 nm; UV (0.01 N HCl) λ_{max} 262 nm (ϵ 9113), λ_{min} 233 nm; UV (0.01 N NaOH) λ_{max} 262 nm (ϵ 7939), λ_{min} 235 nm; NMR (Me_2SO-d_6) δ 3.56 (m, 2 H, 5'-H), 4.47 (m, 1 H, 4'-H), 4.53 (m, 1 H, 2'-H), 5.00 (t, 1 H, 5'-OH, D_2O exchangeable), 5.14 [t (overlap dd), 1 H, methylidene-H_A, J = 2.0 Hz], 5.18 [t (overlap dd), 1 H, methylidene-H_B, J = 2.1 Hz], 5.64 (d, 1 H, 5-H, J = 8.0 Hz), 5.69 (d, 1 H, 1'-H, J = 2.0 Hz), 5.80 (d, 1 H, 3'-OH, D_2O exchangeable), 7.84 (d, 1 H, 6-H, J = 8.0 Hz), 11.5 (s, 1 H, 3-NH, D_2O exchangeable); MS m/z 241 (M^+ + 1), 129 [$C_6H_9O_3$ (sugar residue)], 113 (uracil + 1). Anal. ($C_{10}H_{12}N_2O_5$·0.5H_2O) C, H, N.

1-(2,5-Di-*O*-trityl-3-deoxy-3-methylidene-β-D-ribofuranosyl)-4-(1,2,4-triazol-1-yl)-1*H*-pyrimidin-2-one (29). Compound **27** (2.4 g, 3.3 mmol) and 1,2,4-triazole (3.0 g, 43.4 mmol) were dissolved in 20 mL of pyridine. While the solution was being stirred in an ice/water bath, 4-chlorophenyl phosphorodichloridate (2.4 mL, 14.7 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 days, after which it was evaporated in vacuo to dryness. The residue was dissolved in 50 mL of CH_2Cl_2 and washed with water (2 × 30 mL) and 50% $NaHCO_3$ solution. The CH_2Cl_2 layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to a small volume and purified by silica gel chromatography (eluted with EtOAc) to afford 1.8 g (72%) of product as a white foam: R_f 0.90 (EtOAc); NMR ($CDCl_3$) δ 3.41 (d, 2 H, 5'-H), 4.24 (m, 1 H, 4'-H), 4.70–4.80 (m, 3 H, 2'-H and methylidene), 6.32 (d, 1 H, 5-H), 6.44 (d, 1 H, 1'-H), 7.16–7.36 (m, 30 H, Ar H), 7.76 (s, 1 H, triazolyl 3-H), 8.20 (d, 1 H, 6-H), 9.20 (s, 1 H, triazolyl 5-H). Anal. ($C_{50}H_{41}N_5O_4$) C, H, N.

2',5'-Di-*O*-trityl-3'-deoxy-3'-methylidenecytidine (30). Compound **29** (1.6 g, 2.1 mmol) was dissolved in 40 mL of NH_4OH/dioxane (1:3, v/v) and stirred overnight at room temperature. The solution was then concentrated in vacuo to dryness and the resultant residue was purified on a silica gel column (EtOAc/MeOH, 4:1, v/v, R_f 0.81) to afford 1.3 g (86%) of product as a white foam: NMR ($CDCl_3$) δ 3.29 (d, 2 H, 5'-H), 4.30 (m, 1 H, 4'-H), 4.60–4.80 (m, 3 H, 2'-H and methylidene), 5.20 (d, 1 H, 5-H), 5.41 (d, 1 H, 1'-H), 7.10–7.30 (m, 30 H, Ar H), 7.32 (s, 2 H, 4-NH_2, D_2O exchangeable), 7.81 (d, 1 H, 6-H). Anal. ($C_{48}H_{41}N_3O_4$) C, H, N.

3'-Deoxy-3'-methylidenecytidine (31) and Its Hydrochloride Salt. A suspension of compound **30** (1.2 g, 1.7 mmol) in 20 mL of 80% acetic acid was heated with stirring at 110–115 °C for 15 min, and the resultant solution was diluted with water (20 mL). The reaction mixture was cooled on an ice/bath for 1 h and the precipitated solid was filtered and washed with water. The combined filtrate and washings were evaporated in vacuo to give a glass, which was then purified by silica gel column chromatography to yield 0.35 g (79%) of product as a white foam: R_f 0.50 (CH_2Cl_2/MeOH, 2:1, v/v); UV (MeOH) λ_{max} 272 nm (ϵ 13202), λ_{min} 251 nm; UV (0.01 N HCl) λ_{max} 280 nm (ϵ 15500), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ϵ 10424), λ_{min} 250 nm; NMR (Me_2SO-d_6) δ 3.15 (br, 2 H, 2'-OH and 5'-OH, D_2O exchangeable), 3.61 (m, 2 H, 5'-H), 4.50 (m, 2 H, 2'-H and 4'-H), 5.11 [t (overlap dd), 1 H, methylidene-H_A, J = 2.0 Hz], 5.16 [t (overlap dd), 1 H, methylidene-H_B, J = 2.0 Hz], 5.68 (d, 1 H, 1'-H, J = 6.8 Hz), 5.75 (d, 1 H, 5-H, J = 7.4 Hz), 7.20 (d, 2 H, 4-NH_2, D_2O exchangeable), 7.73 (d, 1 H, 6-H, J = 7.5 Hz); MS m/z 240 (M^+ + 1), 129 [$C_6H_9O_3$ (sugar residue)], 112 (cytosine + 1).

The hydrochloride salt of compound **31** was prepared similarly as described previously and isolated as white crystals: mp 199 °C dec; UV (MeOH) λ_{max} 274 nm (ϵ 13233), λ_{min} 251 nm; UV (0.01 N HCl) λ_{max} 282 nm (ϵ 15523), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 274 nm (ϵ 10476), λ_{min} 253 nm; NMR (Me_2SO-d_6) δ 3.45 (br, 1 H, 5'-OH, D_2O exchangeable), 3.61 (m, 2 H, 5'-H), 4.42 (m, 1 H, 4'-H), 4.61 (m, 1 H, 2'-H), 5.17 (d, 1 H, methylidene-H_A, J = 2.0 Hz), 5.24 [t (overlap dd), 1 H, methylidene-H_B, J = 1.9 Hz], 5.68 (d, 1 H, 1'-H, J = 6.1 Hz), 5.92 (br s, 1 H, 2'-OH, D_2O exchangeable), 6.19 (d, 1 H, 5-H, J = 7.8 Hz), 8.20 (d, 1 H, 6-H, J = 7.8 Hz), 8.72 and 9.31 (two s, 2 H, 4-NH_2, D_2O exchangeable). Anal. ($C_{10}H_{14}ClN_3O_4$) C, H, N.

Single-Crystal X-ray Analysis of 2'-Deoxy-2'-methylidenecytidine Hydrochloride (23·HCl). Colorless crystals of 2'-deoxy-2'-methylidenecytidine hydrochloride (**23**·HCl) were obtained from ethanol. They belong to the monoclinic space group $P2_1$ and have the following cell dimensions: a = 6.0544 (3), b = 12.1720 (5), and c = 8.3088 (5) Å, β = 98.128 (4)°. Three-dimensional X-ray intensity data were measured on a Picker diffractometer with Cu Kα radiation. Of the 1011 unique reflections ($2\theta \leq 125°$), 1006 had intensities $\geq 2.5\sigma(I)$ and were considered observed. The intensities were corrected for Lorentz and polarization factors; no absorption corrections were applied (μ = 29.5 cm^{-1}). The crystal structure was determined by direct methods.[32] Atomic parameters were refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen atoms. Hydrogen atoms were located on difference Fourier maps and refined with isotropic temperature factors. The refinement converged at R = 0.031 and R_w = 0.035 for 1006 observed reflections. A final difference Fourier map showed no significant features.

All calculations were performed with the NRCVAX system of programs.[33] Figure 1 was drawn with the ORTEP program of Johnson.[34]

Biological Test Procedures. Anticancer Test Procedures in Vitro. Anticancer activity was assessed by growth inhibition studies using murine L1210 leukemia, P388 leukemia, Sarcoma 180, and human CCRF-CEM lymphoblastic leukemia cells as described below: Murine L1210, P388, and S-180 cells were maintained as suspension cultures in Fisher's medium and CCRF-CEM cells were maintained as a suspension culture in Roswell Park Memorial Institute medium, both media supplemented with 10% horse serum and all cells maintained at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Under these conditions, the generation time for L1210, P388, S-180, and CCRF-CEM cells is approximately 12, 12, 18, and 20 h, respectively. Each compound, at various concentrations, was added to L1210, P388, S-180, and CCRF-CEM cells (2 × 10^4 cells/mL) in their exponential phase of growth. The cell number of the drug-free cultures (control), as well as that of the cultures supplemented with the tested compounds, were determined after 24, 48, and 72 h of growth.

Anticancer Test Procedures in Vivo. Female BALB/c × DBA/2 F_1 (hereafter called CD_2F_1) mice, obtained from NIH, were used at 8–12 weeks of age. Compounds 2'-deoxy-2'-methylidene-5-fluorocytidine (**22**) and 2'-deoxy-2'-methylidenecytidine hydrochloride (**23**·HCl) were synthesized according to the methods previously described in this paper. 1-β-D-Arabinofuranosylcytosine (ara-C) was obtained from Sigma Chemical Company, St. Louis, MO. Solutions of **22**, **23**, and ara-C in 0.01 M sodium citrate buffer (pH 4.0) were prepared prior to injection and used immediately. Transplantation of L1210 ascites cell form leukemia was carried out every 8–10 weeks by withdrawing peritoneal fluid from donor CD_2F_1 mice bearing 7-day growths. The suspension was centrifuged for 5 min (1200 g), the supernatant peritoneal fluid was decanted, and a 10-fold dilution was made with Fisher's medium supplemented with 10% horse serum. The cell number was determined with a Coulter particle counter and the cell population was adjusted to 10^6 cells/mL: 1 × 10^5 cells/mL were seeded in 10 mL of horse serum/Fisher's medium, [1:9], and once again maintained in culture. For the assay, each mouse received an intraperitoneal injection of a 0.1-mL L1210 cell suspension containing 10^5 cells. The test compounds were administered by intraperitoneal injection beginning 24 h after tumor implantation, twice daily for 6 consecutive days. The drugs were injected as solutions in 0.1 M sodium citrate buffer in a volume of 0.25 mL. The mice were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water ad libitum. Control tumor-bearing mice, given comparable volumes of the vehicle (sodium citrate buffer), were also included. Mice

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were weighed during the course of the experiment and the percentage change of body weight and lethality was used as an indication of drug toxicity. The sensitivity of ascitic neoplasms to these agents was based on the increase in median survival time and the number of long-term (>60 days) survivors achieved by the different treatments.

Procedures of Biochemical Studies. The procedure for extraction of cytidine/deoxycytidine deaminase up to the thymidine affinity column was performed as described.[17] Subsequently, the enzyme preparation was eluted in the void volume of a Blue Sepharose CL-6B column equilibrated with buffer (0.2 M Tris-HCl, pH 7.5, 2 mM dithiothreitol, and 10% glycerol). The fractions containing deaminase activity were pooled and applied to a DE-52 anion exchange column. The purified enzyme, which eluted in a gradient of increasing ionic strength (0 to 0.5 M KCl), was desalted by using G-25 Sephadex before use.

The procedure for quantitating the activity of dCyd deaminase using [2-^{14}C]-dCyd as substrate has been described.[17] The kinetic analysis was performed by using three fixed concentrations of radiolabeled dCyd against four concentrations of each analogue. Inhibition was competitive. The data were fit to the rate equation for competitive inhibition using a nonlinear least-squares computer program.

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Supplementary Material Available: Tables V-IX of the final atomic parameters, anisotropic thermal parameters, bond lengths and angles, torsion angles, and details of hydrogen bonds in the crystal structure of compound **23**·HCl (5 pages). Ordering information is given on any current masthead page.

Synthesis, Stereochemistry, and Biological Activity of the 1-(1-Phenyl-2-methylcyclohexyl)piperidines and the 1-(1-Phenyl-4-methylcyclohexyl)piperidines. Absolute Configuration of the Potent *trans*-(−)-1-(1-Phenyl-2-methylcyclohexyl)piperidine†

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The (−)- and (+)-isomers of the *cis*- and *trans*-Ph/Me 1-(1-phenyl-2-methylcyclohexyl)piperidines have been synthesized and the achiral *cis*- and *trans*-Ph/Me 1-(1-phenyl-4-methylcyclohexyl)piperidines were prepared, and their in vitro [displacement of [^{3}H]TCP (1-[1-(2-thienylcyclohexyl)]piperidine) from the PCP (1-(1-phenylcyclohexyl)piperidine) binding site] and in vivo (rotarod assay) activities determined. The 1-(1-phenyl-2-methylcyclohexyl)piperidine isomers were resolved by classical crystallization procedures, through the diastereomeric salts obtained with *d*- and *l*-10-camphorsulfonic acid. The relative stereochemistry of the *cis*- and *trans*-Ph/Me 1-(1-phenyl-2-methylcyclohexyl)piperidines and the achiral *cis*- and *trans*-Ph/Me 1-(1-phenyl-4-methylcyclohexyl)piperidines was established by using ^{13}C and ^{1}H NMR. Both (−)-*trans*-1-(1-phenyl-2-methylcyclohexyl)piperidine ((−)-**2**) and (+)-*trans*-1-(1-phenyl-2-methylcyclohexyl)piperidine ((+)-**2**) were examined by single-crystal X-ray analysis, and the absolute configuration of (−)-**2** was determined to be 1*S*,2*R*. The (−)-**2** was found to be about five times more potent than PCP in vitro and twice as potent in vivo. It is the most potent of all of the simple methyl-substituted cyclohexyl PCP isomers and is among the most potent PCP-like compounds which have been synthesized. It was nine times more potent in vitro and four times more potent in vivo than (+)-**2**. The racemic *cis*-1-(1-phenyl-2-methylcyclohexyl)piperidine (**3**), and its enantiomers ((+)-**3** and (−)-**3**), were essentially inactive in vitro and in vivo. The *cis*-Ph/Me 1-(1-phenyl-4-methylcyclohexyl)piperidine (**18**) was more potent than *trans*-Ph/Me 1-(1-phenyl-4-methylcyclohexyl)piperidine (**17**), but considerably less potent than (−)-**2**. The enantioselectivity observed at the PCP binding site for (−)-**2** could indicate that this site can discriminate between enantiotopic edges of the achiral PCP (choosing the *pro*-1-*S* edge), as does the μ-opioid receptor in the prodine series of opioids. Benzimidoyl or benzoyl group replacement of the phenyl ring in the 1-(1-phenyl-2-methylcyclohexyl)piperidine series gave compounds which showed little in vitro and in vivo activity.

Several studies have described stereoselective, saturable phencyclidine (1, 1-(1-phenylcyclohexyl)piperidine, PCP) binding sites in the brain of animals of many species.[1-4] PCP has a wide spectrum of activity which directly or indirectly involves dopaminergic and cholinergic neurotransmitters, as well as excitatory amino acid neurotrans-

† Dedicated to Prof. G. B. Marini Bettolo on the occasion of his 75th birthday.

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