

Synthesis and Anticancer and Antiviral Activities of Various 2'- and 3'-Methylidene-Substituted Nucleoside Analogues and Crystal Structure of 2'-Deoxy-2'-methylidenecytidine Hydrochloride¹

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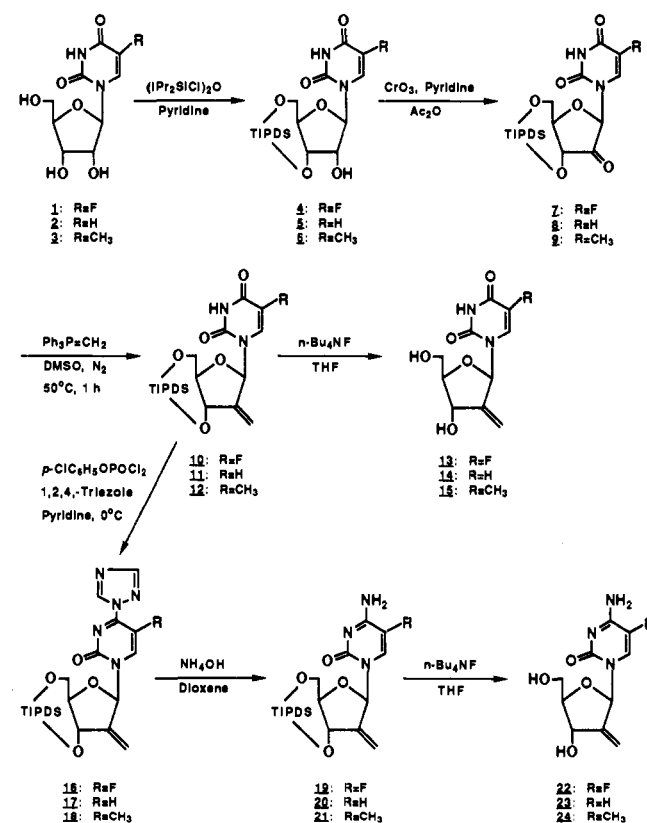
Various 2'- and 3'-methylidene-substituted nucleoside analogues have been synthesized and evaluated as potential anticancer and/or antiviral agents. Among these compounds, 2'-deoxy-2'-methylidene-5-fluorocytidine (22) and 2'-deoxy-2'-methylidenecytidine (23) not only demonstrated potent anticancer activity in culture against murine L1210 and P388 leukemias, Sarcoma 180, and human CCRF-CEM lymphoblastic leukemia, producing ED₅₀ 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 × 100 values and some long-term survivors (>60 days). In addition, compounds 22 and 23 were found to be, 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 anticancer² and antiviral³ 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 deamination⁴⁻⁶ 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

Scheme I^a



^a TIPDS = 1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl.

activities as well as some of the biochemical studies of these compounds and the X-ray crystallographic analysis of

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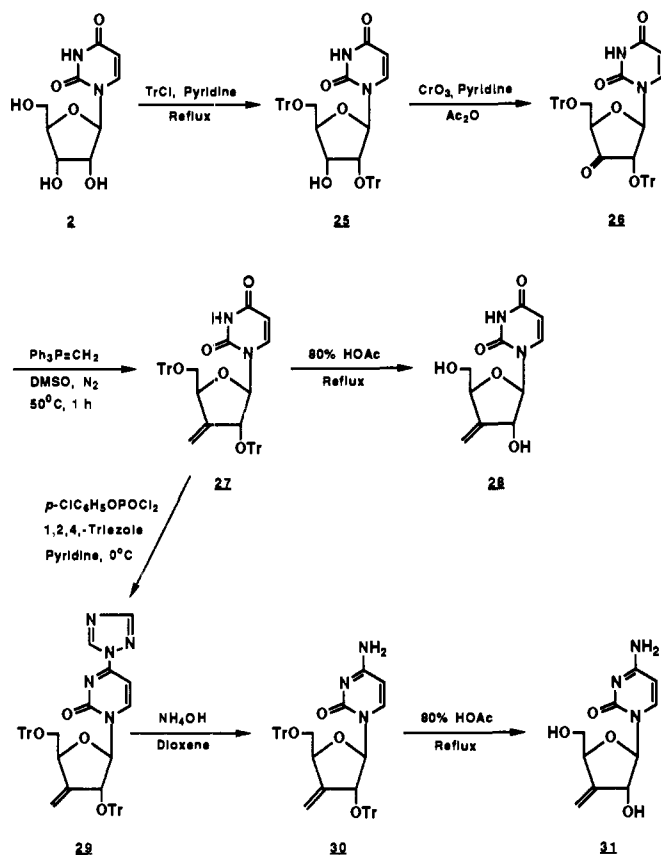
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(1) Inquiries regarding X-ray crystallography should be addressed to G. I. Birnbaum.

(2) Cohen, S. S. Introduction to the Biochemistry of D-Arabinosyl Nucleosides. *Prog. Nucleic Acid Res. Mol. Biol.* 1966, 5, 1-88.

Scheme II



2'-deoxy-2'-methylidene-5-fluorouridine hydrochloride (23-HCl) are described.

- Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. Nucleosides. 110. Synthesis and Antiherpes Virus Activity of Some 2'-Fluoro-2'-deoxyarabinofuranosylpyrimidine Nucleosides. *J. Med. Chem.* 1979, 22, 21-24.
- Creasy, W. A.; Papac, R. J.; Markiw, M. E.; Calabresi, P.; Welch, A. D. Biochemical and Pharmacological Studies with 1- β -D-Arabinofuranosylcytosine in Man. *Biochem. Pharmacol.* 1966, 15, 1417-1428.
- Camiener, G. W. Studies of the Enzymatic Deamination of Cytosine Arabinoside-III. Substrate Requirements and Inhibitors of the Deaminase of Human Liver. *Biochem. Pharmacol.* 1967, 16, 1691-1702.
- Wright, J. A.; Wilson, D. P.; Fox, J. J. Nucleosides. LXIV. Fluoro Sugar Analogs of Arabinosyl- and Xylosylcytosines. *J. Med. Chem.* 1970, 13, 269-272.
- Bobek, M.; Cheng, Y. C.; Bloch, A. Novel Arabinofuranosyl Derivatives of Cytosine Resistant to Enzymatic Deamination and Possessing Potent Antitumor Activity. *J. Med. Chem.* 1978, 21, 597-598.
- Chou, T. C.; Burchenal, J. H.; Fox, J. J.; Watanabe, K. A.; Chu, C. K.; Phillips, F. S. Metabolism and Effects of 5-(β -D-Ribofuranosyl)isocytosine in P815 Cells. *Cancer Res.* 1979, 39, 720-728.
- Mian, A. M.; Long, R. A.; Allen, L. B.; Sidwell, R. W.; Robins, R. K.; Khwaja, T. A. Synthesis and Antitumor and Antiviral Activities of 1- β -D-Arabinofuranosyl-2-amino-1,4(2H)-imino-pyrimidine and Its Derivatives. *J. Med. Chem.* 1979, 22, 514-518.
- Rosowsky, A.; Kim, S. H.; Ross, J.; Wick, M. M. Lipophilic 5'-(Alkyl phosphate) Esters of 1- β -D-Arabinofuranosylcytosine and Its N^4 -Acyl and 2,2'-Anhydro-3'-O-acyl Derivatives as Potential Prodrugs. *J. Med. Chem.* 1982, 25, 171-178.
- Chwang, T. L.; Fridland, A.; Avery, T. L. 2'-O-Nitro-1- β -D-Arabinofuranosylcytosine. A New Derivative of 1- β -D-Arabinofuranosylcytosine That Resists Enzymatic Deamination and Has Antileukemic Activity. *J. Med. Chem.* 1983, 26, 280-283.

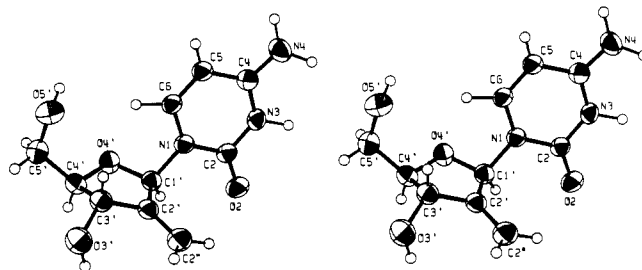


Figure 1. Stereospecific view of the 2'-deoxy-2'-methylidene-5-fluorouridine hydrochloride molecule.

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'-methylidene-5-methylcytosine (24) were synthesized as depicted in Scheme I. Treatment¹⁹ of 5-fluorouridine (1), uridine (2), and 5-methyluridine (3) with 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane in pyridine gave the corresponding 3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl) derivatives 4, 5, and 6. Oxidation²⁰ 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 reaction²¹ with methylenetriphenylphosphorane in anhydrous Me_2SO at 50°C under nitrogen. Deprotection¹⁹ of compounds 10-12 with tetra-*n*-butylammonium fluoride in THF gave the desired products 13, 14, and 15, respectively. Treatment^{12,22} of

- Lin, T. S.; Gao, Y. S.; Mancini, W. R. Synthesis and Biological Activity of Various 3'-Azido and 3'-Amino Analogues of 5-Substituted Pyrimidine Deoxyribonucleosides. *J. Med. Chem.* 1983, 26, 1691-1696.
- Lin, T. S.; Mancini, W. R. Synthesis and Antineoplastic Activity of 3'-Azido and 3'-Amino Analogues of Pyrimidine Deoxyribonucleosides. *J. Med. Chem.* 1983, 26, 544-548.
- Beisler, J. A.; Abbasi, M. M.; Driscoll, J. S. The Synthesis and Antitumor Activity of Arabinosyl-5-azacytosine. *Biochem. Pharmacol.* 1977, 26, 2469-2472.
- Bobek, M.; Kawai, I.; Sharma, R. A.; Grill, S.; Dutschman, G.; Cheng, Y. C. Acetylenic Nucleosides. 4. 1- β -D-Arabinofuranosyl-5-ethynylcytosine. Improved Synthesis and Evaluation of Biochemical and Antiviral Properties. *J. Med. Chem.* 1987, 30, 2154-2157.
- Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamagami, K. Design, Synthesis, and Antineoplastic Activity of 2'-Deoxy-2'-methylidene-5-azacytosine. *J. Med. Chem.* 1988, 31, 1063-1064.
- Mancini, W. R.; Lin, T. S. Ribo- and Deoxyribonucleoside Effect on 3'-Amino-2',3'-dideoxycytidine-Induced Cytotoxicity in Cultured L1210 Cells. *Biochem. Pharmacol.* 1983, 32, 2427-2432.
- Mancini, W. R.; Williams, M. S.; Lin, T. S. Specific Inhibition of DNA Biosynthesis Induced by 3'-Amino-2',3'-dideoxycytidine. *Biochemistry* 1988, 27, 8832-8839.
- Robins, M. J.; Wilson, J. S.; Hansske, F. Nucleic Acid Related Compounds. 42. A General Procedure for the Efficient Deoxygenation of Secondary Alcohols. Regiospecific and Stereoselective Conversion of Ribonucleosides to 2'-Deoxy-nucleosides. *J. Am. Chem. Soc.* 1983, 105, 4059-4065.
- Hansske, F.; Madej, D.; Robins, M. J. 2'- and 3'-Keto-nucleosides and Their Arabinosyl and Xylo Reduction Products. Convenient Access Via Selective Protection and Oxidation of Ribonucleosides. *Tetrahedron* 1984, 40, 125-135.
- Greenwald, R.; Chaykovsky, M.; Corey, E. J. The Wittig Reaction Using Methylsulfinyl Carbanion-Dimethyl Sulfoxide. *J. Org. Chem.* 1963, 28, 1128-1129.

compounds 10–12 with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature yielded the 4-triazolypyrimidinone derivatives 16–18. Subsequent treatment¹² of 16–18 with a mixture of ammonium hydroxide/dioxane (1:3) produced the corresponding cytidine derivatives 19–21, which were then deblocked by reaction¹⁹ 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'-methylidene-2'-methylideneuridine (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. Oxidation²⁰ of 25 with CrO₃/pyridine/Ac₂O yielded the 3'-carbonyl compound 26, which was then converted to the corresponding 3'-methylidene derivative by Wittig reaction²¹ 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-triazolypyrimidinone derivative 29. Subsequent treatment¹² of 29 with aqueous ammonia in dioxane yielded the protected 3'-deoxy-3'-methylidene-2'-methylidene-2'-methylideneuridine 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'-methylidene-2'-methylideneuridine 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 sp² hybridization of C2' causes a flattening of the furanose ring. The maximum degree of pucker²⁴ $\tau_m = 31.7^\circ$, not very much lower than the usual 37°. The phase angle of pseudorotation $P = 42.3^\circ$ is somewhat outside normal limits and indicates a C4' exo/C3' endo (₄T⁸) pucker. The CH₂OH side chain adopts the common gauche⁺ conformation and the conformation about the glycosidic bond (C6-N1-C1'-O4') is anti ($\chi_{CN} = 31.2^\circ$).

The esds of bond distances and angles are 0.003–0.004 Å and 0.2–0.3°, respectively. In the sugar ring, the sp² 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 ED₅₀ 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

| compd | ED ₅₀ ^a values in μM against cell lines | | | | CCRF-CEM (dCK ⁻) ^b |
|-------|---|------|-------|----------|---|
| | L1210 | P388 | S-180 | CCRF-CEM | |
| 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 |

^aThe ED₅₀ 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. ^bCCRF-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% (ED₅₀) 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'-methylidene-2'-methylidene-2'-methylidene-5-fluorocytidine (22) and 2'-deoxy-2'-methylidene-2'-methylidene-5-fluorocytidine (23), produced the greatest activity among the synthesized compounds, with respective ED₅₀ 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'-methylidene-2'-methylidene-2'-methylidene-5-fluorocytidine (23) to have an ED₅₀ of 0.40 μM for inhibition of L1210 cells in vitro. Whereas the ED₅₀ 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 ED₅₀ values

- (22) Sung, W. L. Chemical Conversion of Thymidine into 5-Methyl-2'-deoxycytidine. *J. Chem. Soc., Chem. Commun.* 1981, No. 19705, 1089.
 (23) Cook, A. F.; Moffatt, J. G. Carbodiimide-Sulfoxide Reactions. VI. Synthesis of 2'- and 3'-Ketouridines. *J. Am. Chem. Soc.* 1967, 89, 2697-2705.
 (24) Berman, H. M. Conformational Principles of Nucleic Acids. In *Topics in Nucleic Acid Structure*; Neidle, S., Ed.; Macmillan: London, 1981; pp 1-15.
 (25) Taylor, R.; Kennard, O. Molecular Structures of Nucleosides and Nucleotides. 2. Orthogonal Coordinates for Standard Nucleic Acid Base Residues. *J. Am. Chem. Soc.* 1982, 104, 3209-3212.

- (26) Birnbaum, G. I.; Shugar, D. Biologically Active Nucleosides and Nucleotides: Conformational Features and Interactions with Enzymes. In *Topics in Nucleic Acid Structure*; Neidle, S., Ed.; Macmillan: London, 1987; Part 3, pp 1-70.
 (27) Birnbaum, G. I.; Deslauriers, R.; Lin, T. S.; Shiau, G. T.; Prusoff, W. H. A Novel Intramolecular Hydrogen Bond in the Crystal Structure of 5-(Hydroxymethyl)-2'-deoxyuridine, an Antiviral and Antineoplastic Nucleoside. Conformational Analysis of the Deoxyribose Ring. *J. Am. Chem. Soc.* 1980, 102, 4236-4241.

Table II. Effects of 2'-Deoxy-2'-methylidene-5-fluorocytidine (22), 2'-Deoxy-2'-methylidene-5-fluorocytidine (23), and 1- β -D-Arabinofuranosylcytosine (ara-C) on the Survival of CD₂F₁ 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 |

^aDrugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, twice daily for 6 consecutive days. ^bDeaths due to toxicity occurring at a maximum body weight decrease of 10 to 22% from the average post injection body weight. ^cAverage 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 $\times 100$. The average survival time of the untreated tumor-bearing control animals was 7.6 days. ^eLong-term survivors are the number of mice that survived for >60 days relative to the number of treated mice.

of 22 μ M against L1210, 6.5 μ M against S-180, and 30 μ M 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 ED₅₀ values of 10 and 22 μ M, respectively. Conversely, 2'-deoxy-2'-methylideneuridine (14), 3'-deoxy-3'-methylideneuridine (28), and 3'-deoxy-3'-methylidene-5-fluorocytidine (31) showed no activity at concentrations up to 100 μ M.

Compounds 22 and 23 were also examined against CCRF-CEM deoxycytidine kinase deficient variant [CCRF-CEM (dCK⁻)] and ED₅₀ 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'-methylidene-5-fluorocytidine (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'-methylidene-5-fluorocytidine series in vitro, 2'-deoxy-2'-methylidene-5-fluorocytidine (22) and 2'-deoxy-2'-methylidene-5-fluorocytidine (23), along with ara-C for comparison, were screened against L1210 leukemia bearing CD₂F₁ 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'-methylidene-5-fluorocytidine (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 |

^aID₉₀ represents the drug concentration (μM) required to inhibit 90% of virus yield during a 24-h growth period. ^b80% 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 |

^aThe 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 (r² = 0.99855).

period was designated as ID₉₀. 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'-methylidene-2'-deoxycytidine (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'-methylidene-2'-methylidene-5-fluorocytidine (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 Me₄Si 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-Tetraisopropylidisiloxane-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-tetraisopropylidisiloxane (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 MgSO₄ and filtered. The filtrate was then reduced to a small volume in vacuo and chromatographed on a silica gel column (CHCl₃/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 (CDCl₃) δ 1.05-1.15 [m, 28 H, CH(CH₃)₂], 4.44-4.75 (m, 6 H, 2'-H, 3'-H, 4'-H, and 5'-H; and 2'-OH, D₂O exchangeable), 5.50 (d, 1 H, 1'-H), 7.35 (d, 1 H, 6-H). Anal. (C₂₁H₃₇N₂O₇Si₂F) C, H, N.

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)uridine (5).¹⁹ yield 10.6 g (76%), as a foam; TLC, R_f 0.76 (CH₂Cl₂/MeOH, 10:1, v/v); NMR (CDCl₃) δ 1.10-1.20 [m, 28 H, CH(CH₃)₂], 4.40-4.70 (m, 6 H, 2'-H, 3'-H, 4'-H, 5'-H; and 2'-OH, D₂O ex-

- (28) Cheng, Y. C.; Huang, E. S.; Lin, J. C.; Mar, E. C.; Pagano, J. S.; Dutschman, G. E.; Grill, S. P. Unique Spectrum of Activity of 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine Against Herpesviruses *in vitro* and Its Mode of Action Against Herpes Simplex Virus Type 1. *Proc. Natl. Acad. Sci. U.S.A.* 1983, 80, 2767-2770.
- (29) Camlener, G. W.; Smith, C. G. Studies of the Enzymatic Deamination of Cytosine Arabinoside-I. Enzyme Distribution and Species Specificity. *Biochem. Pharmacol.* 1965, 14, 1405-1416.
- (30) Chabner, B. A.; Johns, D. G.; Coleman, C. N.; Drake, J. C.; Evans, W. H. Purification and Properties of Cytidine Deaminase from Normal and Leukemic Granulocytes. *J. Clin. Invest.* 1974, 53, 922-931.

- (31) Chidgeavazde, Z. G.; Beabealashvili, R. Sh.; Atrazhev, A. M.; Kukhanova, M. K.; Azhaye, A. V.; Krayevshy, A. A. 2',3'-Dideoxy-3'-aminonucleoside-5'-Triphosphates are the Terminators of DNA Synthesis Catalyzed by DNA Polymerases. *Nucleic Acids Res.* 1984, 12, 1671-1686.

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, D₂O exchangeable).

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-5-methyluridine (6): yield 1.6 g (69%), as a foam; TLC, *R_f* 0.77 (CH₂Cl₂/MeOH, 10:1, v/v); NMR (CDCl₃) δ 1.10–1.20 [m, 28 H, CH(CH₃)₂], 2.00 (s, 3 H, 5-CH₃), 3.52 (s, 1 H, 2'-OH, D₂O 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, D₂O exchangeable). Anal. (C₂₂H₄₀N₂O₇Si₂) C, N, H calcd 5.60, found 5.14.

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-2'-keto-5-fluorouridine (7): To a stirred suspension of CrO₃ (0.9 g), pyridine (1.5 mL), and acetic anhydride (0.9 mL) in CH₂Cl₂ (20 mL) was added compound 4 (1.6 g, 3.2 mmol) in CH₂Cl₂ (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 (CH₂Cl₂/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 (CDCl₃) δ 1.05–1.25 [m, 28 H, CH(CH₃)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-Tetraisopropylidisiloxane-1,3-diyl)-2'-keto-uridine (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 (CH₂Cl₂/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 (CDCl₃) δ 0.95–1.15 [m, 28 H, CH(CH₃)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, D₂O exchangeable).

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-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 (CH₂Cl₂/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 (CDCl₃) δ 1.00–1.21 [m, 28 H, CH(CH₃)₂], 2.10 (s, 3 H, 5-CH₃), 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-Tetraisopropylidisiloxane-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 Me₂SO 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 CH₂Cl₂ (3 × 50 mL). The combined CH₂Cl₂ extract was dried with anhydrous MgSO₄ and filtered. The filtrate was then reduced to a small volume in vacuo and chromatographed on a silica gel column (CH₂Cl₂/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 (CDCl₃) δ 0.90–1.10 [m, 28 H, CH(CH₃)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. (C₂₂H₃₇FN₂O₆Si₂) C, H, N.

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-2'-deoxy-2'-methylideneuridine (11): yield 0.90 g (34%) as a white foam; TLC, *R_f* 0.64 (CH₂Cl₂/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 (CDCl₃) δ 0.80–1.00 [m, 28 H, CH(CH₃)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, D₂O exchangeable). Anal. (C₂₂H₃₈N₂O₆Si₂) C, H, N.

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-2'-deoxy-2'-methylidene-5-methyluridine (12): yield 0.9 g (40%) as a white foam; TLC, *R_f* 0.69 (CH₂Cl₂/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 (CDCl₃) δ 1.05–1.20 [m, 28 H, CH(CH₃)₂], 2.10 (s, 3 H, 5-CH₃), 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, D₂O exchangeable). Anal. (C₂₃H₄₀N₂O₆Si₂) 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*-Bu₄N⁺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 CH₂Cl₂ and H₂O. The aqueous phase was evaporated in vacuo to dryness, and the residue was chromatographed on a silica gel column (CH₂Cl₂/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 (CH₂Cl₂/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 (Me₂SO-*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, D₂O 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, D₂O 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, D₂O exchangeable); MS *m/z* 259 (M⁺ + 1), 131 (5-fluorouracil + 1), 129 [C₆H₉O₃ (sugar residue)]. Anal. (C₁₀H₁₁FN₂O₅) 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 10570), 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 10249), λ_{min} 233 nm; NMR (Me₂SO-*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, D₂O 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, D₂O 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, D₂O exchangeable); MS *m/z* 241 (M⁺ + 1), 129 [C₆H₉O₃ (sugar residue)], 113 (uracil + 1). Anal. (C₁₀H₁₂N₂O₅) 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 10396), λ_{min} 237 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ε 10960), λ_{min} 237 nm; NMR (Me₂SO-*d*₆) δ 1.80 (s, 3 H, 5-CH₃), 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, D₂O 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, D₂O exchangeable), 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, D₂O exchangeable); MS *m/z* 255 (M⁺ + 1), 129 [C₆H₉O₃ (sugar residue)], 127 (thymine + 1). Anal. (C₁₁H₁₄N₂O₅) C, H, N.

1-[3,5-O-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-2-deoxy-2-methylidene-β-D-ribofuranosyl]-4-(1,2,4-triazol-1-yl)-1H-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 CH₂Cl₂ (50 mL) and washed with water (4 × 25 mL). The CH₂Cl₂ solution was dried (MgSO₄) 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. (C₂₄H₃₉N₅O₅Si₂) C, H, N.

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-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 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 20:1, v/v); NMR (CDCl_3) δ 0.85–1.10 [m, 28 H, $\text{CH}(\text{CH}_3)_2$], 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-Tetraisopropylidisiloxane-1,3-diyl)-2'-deoxy-2'-methylideneuridine (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 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 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, $\text{CH}(\text{CH}_3)_2$], 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. ($\text{C}_{22}\text{H}_{39}\text{N}_3\text{O}_5\text{Si}_2$) C, H, N.

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxane-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 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 10:1, v/v); NMR (CDCl_3) δ 1.00–1.10 [m, 28 H, $\text{CH}(\text{CH}_3)_2$], 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. ($\text{C}_{23}\text{H}_{41}\text{N}_3\text{O}_5\text{Si}_2$) 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 $n\text{-Bu}_4\text{N}^+\text{F}^-/\text{THF}$ 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, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 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} 278 nm (ϵ 7939), λ_{min} 265 nm; NMR ($\text{Me}_2\text{SO}-d_6$) 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 [$\text{C}_8\text{H}_9\text{O}_3$ (sugar residue)]. Anal. ($\text{C}_{10}\text{H}_{12}\text{FN}_3\text{O}_4$) C, H, N.

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

2'-Deoxy-2'-methylideneuridine (23) and its hydrochloride salt: yield 0.6 g (92%), as a foam; TLC, R_f 0.51 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 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 14541), λ_{min} 246 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ϵ 10412), λ_{min} 250 nm; NMR ($\text{Me}_2\text{SO}-d_6$) 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 [$\text{C}_8\text{H}_9\text{O}_3$ (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 14611), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ϵ 10476), λ_{min} 252 nm; NMR ($\text{Me}_2\text{SO}-d_6$) 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. ($\text{C}_{10}\text{H}_{14}\text{ClN}_3\text{O}_4$) C, H, N.

2'-Deoxy-2'-methylidene-5-methylcytidine (24): yield 0.12 g (60%); mp 199–201 °C; TLC, R_f 0.52 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 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 10716), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 278 nm (ϵ 8326), λ_{min} 258 nm; NMR ($\text{Me}_2\text{SO}-d_6$) 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 [$\text{C}_8\text{H}_9\text{O}_3$ (sugar residue)], 126 (5-methylcytosine + 1). Anal. ($\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_4$) 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 ($\text{CHCl}_3/\text{EtOAc}$, 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. ($\text{C}_{48}\text{H}_{40}\text{N}_2\text{O}_5\text{H}_2\text{O}$) 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.

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 [2-¹⁴C]-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 [³H]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 ¹³C and ¹H 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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- (1) French, E. D.; Pilapip, C.; Quirion, R. *Eur. J. Med. Chem.* 1985, 116, 1.
- (2) Quirion, R.; Hammer, R. P., Jr.; Herkenham, M.; Pert, C. B. *Proc. Natl. Acad. Sci. U.S.A.* 1981, 78, 5881.
- (3) Quirion, R.; Rice, K. C.; Skolnick, P.; Paul, S.; Pert, C. B. *Eur. J. Pharmacol.* 1981, 74, 107.
- (4) Jacobson, A. E.; Harrison, E. A., Jr.; Mattson, M. V.; Rafferty, M. F.; Rice, K. C.; Woods, J. H.; Winger, G.; Solomon, R. E.; Lessor, R.; Silverton, J. V. *J. Pharmacol. Exp. Ther.* 1987, 243, 110.