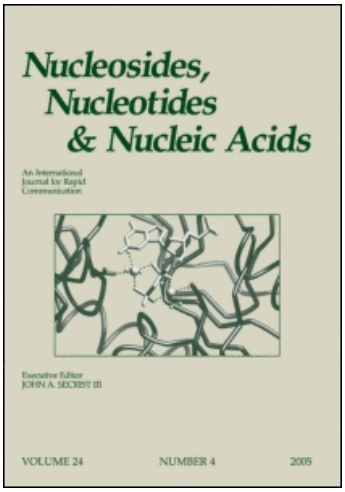

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Synthesis of 2',3'-Dideoxy-2'-methylene Pyrimidine Nucleosides as Potential Anti-Human Immunodeficiency Virus (HIV) Agents

Tai-Shun Lin[a]; Mei-Zhen Luo[a]; Mao-Chin Liu[a]
[a] Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut

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SYNTHESES OF 2',3'-DIDEOXY-2'-METHYLENE PYRIMIDINE NUCLEOSIDES AS POTENTIAL ANTI-HUMAN IMMUNODEFICIENCY VIRUS (HIV) AGENTS

Tai-Shun Lin,* Mei-Zhen Luo, and Mao-Chin Liu

Department of Pharmacology and Comprehensive Cancer Center,
Yale University School of Medicine, New Haven, Connecticut, 06510.

Abstract: Several 2',3'-dideoxy-2'-methylene pyrimidine nucleosides, 2',3'-dideoxy-2'-methylenecytidine hydrochloride (**20**), 2',3'-dideoxy-2'-methyleneuridine (**21**), and 2',3'-dideoxy-2'-methylene-5-methyluridine (**22**), have been synthesized via a multi-step synthesis from uridine and 5-methyluridine, respectively. These compounds were tested for their cytotoxicity against L1210, S-180, CCRF-CEM, and P388 cells in culture and their antiviral activity is under investigation.

Recently, 2'-deoxy-2'-methylenecytidine has been synthesized by Ueda and coworkers,[1-3] Samano and Robins,[4] and our laboratory.[5] 2'-Deoxy-2'-methylenecytidine and its 5-fluoro derivative showed significant antitumor activities in both in vitro[3,5] and in vivo[5,6] studies. Baker et al.[7] have reported that 2'-deoxy-2'-methylenecytidine functioned as an irreversible inhibitor of Escherichia coli ribonucleoside diphosphate reductase. 2',3'-Dideoxy pyrimidine nucleosides, such as 2',3'-dideoxycytidine (D2C),[8] 3'-deoxythymidine (D2T),[8] 2',3'-didehydro-2',3'-dideoxycytidine (D4C),[9-11] and 2',3'-didehydro-3'-deoxythymidine (D4T),[10-14] have been identified as potent and selective inhibitors of human immunodeficiency virus (HIV) replication. Recently, Sharma and Bobek[15] reported the syntheses of several 2',3'-dideoxy-3'-methylene pyrimidine nucleosides as analogues of D4C and D4T with an exocyclic methylene group at C-3' position. In this

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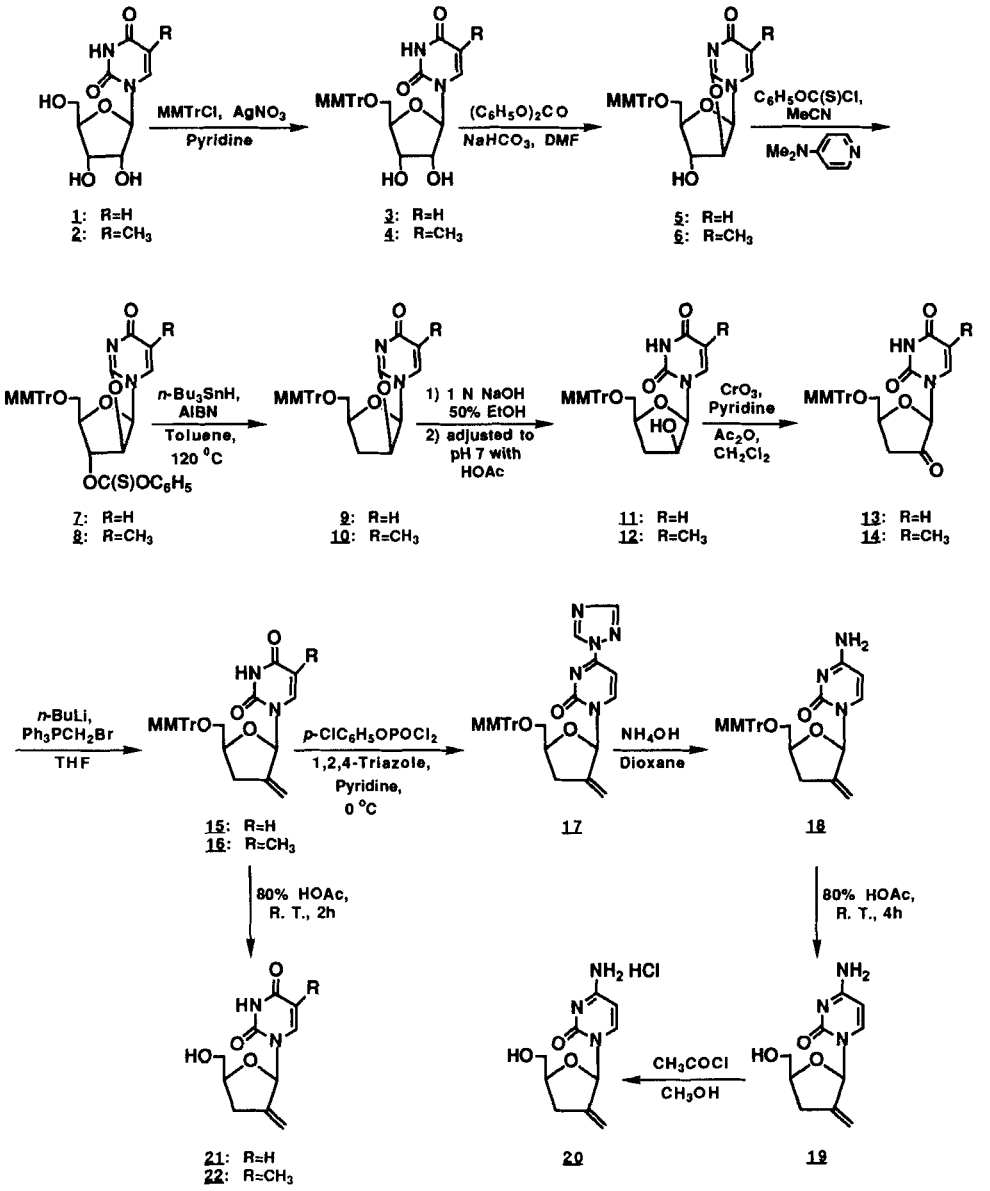
This paper is dedicated to the memory of Professor Tohru Ueda in tribute for the many contributions in chemistry, particularly in nucleoside chemistry, that he had accomplished during his lifetime.

report, we would like to describe the syntheses of several 2',3'-dideoxy-2'-methylene pyrimidine nucleosides, which have shared some of the structural features with both 2'-deoxy-2'-methylenecytidine and 2',3'-dideoxy nucleosides, as potential antiviral and/or anticancer agents.

2',3'-Dideoxy-2'-methylenecytidine (**19**), 2',3'-dideoxy-2'-methyleneuridine (**21**) and 2',3'-dideoxy-2'-methylene-5-methyluridine (**22**) were synthesized as shown in the Scheme. Treatment of uridine (**1**) with 4-methoxytrityl chloride in anhydrous pyridine gave the 5'-O-4-methoxytrityl derivative **3** in 60% yield. Conversely, the synthesis of the 5'-O-4-methoxytrityl derivative of 5-methyluridine derivative **4** was much more difficult. Under the same reaction condition, the yield of compound **4** was only about 10%. However, addition of silver nitrate as a catalyst[16] led to the acceleration of the reaction and the yields of compounds **3** and **4** were increased to 73% and 78%, respectively. Conversion[17] of **3** and **4** to the corresponding 2,2'-anhydro analogues **5** and **6** was accomplished by the respective reaction of compounds **3** and **4** with diphenyl carbonate and sodium bicarbonate in DMF at 150 °C. Treatment[18,19] of compounds **5** and **6** with phenyl chlorothionocarbonate and 4-dimethylaminopyridine in anhydrous acetonitrile at room temperature produced the respective 3'-O-phenoxythiocarbonyl derivatives **7** and **8**. Reduction[18,19] of **7** and **8** with tri-*n*-butyltin hydride (*n*-Bu_3SnH) and azobis(isobutyronitrile) (AIBN) in toluene at reflux temperature yielded the corresponding 3'-deoxy nucleosides **9** and **10**. Treatment of compounds **9** and **10** with 1N NaOH in 50% ethanol, followed by neutralization with acetic acid to pH 7, afforded the arabinosides **11** and **12**, respectively. Oxidation[20] of compounds **11** and **12** with chromium (VI) oxide/pyridine/acetic anhydride complex (1:2:1, molar ratio) in methylene chloride gave the corresponding 2'-ketonucleosides **13** and **14**, which were then converted to the 2'-methylene analogues **15** and **16** by reaction[3] with methyltriphenylphosphonium bromide and *n*-butyllithium in dry THF under nitrogen at room temperature. Treatment[19,21] of compound **15** with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in anhydrous pyridine at room temperature yielded the 4-triazolylpyrimidinone derivative **17**. Subsequent treatment of of compound **17** with a mixture of ammonium hydroxide and dioxane (1:3, v/v) gave the corresponding cytidine derivative **18**, which was then deblocked by reaction with 80% acetic acid at room temperature to afford the target 2',3'-dideoxycytidine analogue **19**. Treatment of compound **19** with acetyl chloride in methanol produced the hydrochloride salt **20** as white crystals. Treatment of compounds **15** and **16** with 80% acetic acid at room temperature for 2 h furnished the corresponding final products **21** and **22**.

Originally, 2',3'-dideoxy-2'-methylene-5'-O-trityluridine[22] was successfully synthesized from 5'-O-trityluridine by the same synthetic route as previously described.



1: R=H
2: R=CH_3

3: R=H
4: R=CH_3

5: R=H
6: R=CH_3

7: R=H
8: R=CH_3

9: R=H
10: R=CH_3

11: R=H
12: R=CH_3

13: R=H
14: R=CH_3

15: R=H
16: R=CH_3

17

18

21: R=H
22: R=CH_3

20

19

MMTrCl = 4-methoxytrityl chloride

Scheme

However, deblocking of the 5'-O-trityl group in this compound by reaction with 80% acetic acid at an elevated temperature led to the cleavage of the glycosyl bond.

Compounds **20**, **21**, and **22** were tested for their cytotoxicity against L1210, S-180, CCRF-CEM, and P388 cell lines in culture, producing the corresponding ED_{50} values of 300, >500, 150, and 125 μM; 60, >100, 70, and 30 μM; and 200, >500, 400, and 150 μM. In general, the P388 and S-180 cells were the most and the least sensitive to these compounds, respectively. Compound **21** was more cytotoxic to these cell lines than either compound **20** or compound **22**.

Compounds **20-22** were evaluated against HIV-1 in H-9 cells and showed no activity up to a concentration of 100 μM.

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 compounds, **19-22**) with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. The mass spectra (at 50 eV) were provided by Yale University Chemical Instrumentation Center. 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.

5'-O-4-Methoxytrityluridine (3). A solution of uridine (**1**, 20.0 g, 82 mmol) and 4-methoxytrityl chloride (27.7 g, 90 mmol) in 200 mL of anhydrous pyridine was stirred overnight at room temperature and then warmed to 65 °C for 4 h. The reaction mixture was evaporated under reduced pressure to dryness. The residue was coevaporated with toluene (2 x 50 mL) under reduced pressure and then dissolved in methylene chloride, washed with water (100 mL), brine (60 mL), and water (60 mL). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was evaporated to dryness under reduced pressure to afford a syrup, which was then chromatographed on a silica gel column (CH_2Cl_2/CH_3OH, 20:1, v/v). The fractions containing the desired product (R_f 0.23) were pooled together and the solvent was removed under reduced pressure to give 25.4 g (43%) of a white solid: mp 116-118 °C; NMR ($CDCl_3$) δ 3.50 (d, 2H, 5'-H), 3.75 (s, 3H, $-OCH_3$), 4.10 (m, 1H, 4'-H), 4.25-4.40 (m, 2H, 2'-H and 3'-H), 5.10-5.20 (m, 2H, 2'-OH and 3'-OH, D_2O exchangeable), 5.30 (d, 1H, 5-H), 5.90 (s, 1H, 1'-H), 6.80-7.40 (m, 14H, ArH), 7.95 (d, 1H, 6-H); UV (CH_3OH) λ_{max} 264 nm (ε 11000), λ_{min} 252 nm. Anal. Calcd. for $C_{29}H_{28}N_2O_7$: C, 67.43; H, 5.46; N, 5.42. Found: C, 67.31; H, 5.69; N, 5.21.

5'-O-4-Methoxytrityl-5-methyluridine (4). A mixture of 5-methyluridine (**2**, 5.0 g, 19.4 mmol), 4-methoxytrityl chloride (6.6 g, 21.3 mmol) and silver nitrate (3.3 g, 19.4 mmol) in 100 mL of anhydrous pyridine was stirred overnight at room temperature and then heated at 65 °C for 12 h. The cooled reaction mixture was evaporated in vacuo to dryness, coevaporated with toluene (2 x 50 mL) and stirred with CH_2Cl_2/CH_3OH (2:1, v/v, 120 mL) for 1 h. The mixture was filtered and washed with CH_2Cl_2 (20 mL). The combined filtrate and washings were washed with water (50 mL), brine (20 mL), and again with water (20 mL). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was evaporated to dryness under reduced pressure to afford a syrup, which was then chromatographed on a silica gel column (CH_2Cl_2/CH_3OH, 20:1, v/v). The fractions containing the desired product (R_f 0.32) were pooled and the solvent was removed under reduced pressure to give 8.0 g (78%) of product as a white solid: mp 122-124 °C; NMR ($CDCl_3$) δ 1.45 (s, 3H, 5-CH_3), 3.50 (m, 2H, 5'-H), 3.80 (s, 3H, -OCH_3), 4.25 (m, 1H, 4'-H), 4.40-4.50 (m, 2H, 2'-H and 3'-H), 5.36-5.60 (m, 2H, 2'-OH and 3'-OH, D_2O exchangeable), 5.95 (s, 1H, 1'-H), 6.80-7.40 (m, 14H, ArH), 7.70 (d, 1H, 6-H), 10.5 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 268 nm (ε 9600), λ_{min} 252 nm. Anal. Calcd. for $C_{30}H_{30}N_2O_7$: C, 67.91; H, 5.70; N, 5.28. Found: C, 68.20; H, 5.96; N, 5.58.

2,2'-Anhydro-1-(5-O-4-methoxytrityl-β-D-arabinofuranosyl)-uracil (5). A mixture of compound **3** (6.0 g, 11.6 mmol), diphenyl carbonate (4.1 g, 19.3 mmol), and sodium bicarbonate (0.12 g) in 15 mL of dry DMF was heated at 125 °C with stirring until evolution of carbon dioxide ceased (~ 90 min). The reaction mixture was cooled and then poured into 100 mL of ether with stirring to give a gum, which was solidified when triturated with ether. The solid was filtered, washed with ether, and crystallized from EtOH to yield 5.5 g (94%) of product as white crystal: mp 169-170 °C; TLC, R_f 0.50 (CH_2Cl_2/CH_3OH, 10:1, v/v); NMR ($CDCl_3$) δ 2.80-3.10 (m, 3H, 3'-H and 5'-H), 3.65 (s, 3H, -OCH_3), 4.48 (m, 1H, 4'-H), 5.25 (m, 1H, 2'-H), 5.37 (d, 1H, 3'-OH, D_2O exchangeable), 5.80 (d, 1H, 5-H), 6.18 (d, 1H, 1'-H), 6.80-7.25 (m, 15H, ArH and 6-H); UV (CH_3OH) λ_{max} 234 nm (ε 19700), and shoulder at 250 nm (ε 10200). Anal. Calcd. for $C_{29}H_{26}N_2O_6$: C, 69.86; H, 5.26; N, 5.62. Found: C, 69.57; H, 5.01; N, 5.42.

2,2'-Anhydro-1-(5-O-4-methoxytrityl-β-D-arabinofuranosyl)-5-methyluracil (6). This compound was synthesized from compound **4** as described for the synthesis of compound **5**: yield, 2.5 g (65%); mp 88-90 °C; TLC, R_f 0.53

(CH_2Cl_2/CH_3OH, 10:1, v/v); NMR ($CDCl_3$) δ 1.80 (s, 3H, 5-CH_3), 2.90 (m, 2H, 5'-H), 3.70 (s, 3H, -OCH_3), 4.40-4.50 (m, 2H, 3'-H and 4'-H), 5.30 (t, 1H, 2'-H), 5.57 (br s, 1H, 3'-OH, D_2O exchangeable), 6.20 (d, 1H, 1'-H), 6.80-7.30 (m, 15H, ArH and 6-H); UV (CH_3OH) λ_{max} 234 nm (ε 18900), and shoulder at 248 nm (ε 11100). Anal. Calcd. for $C_{30}H_{28}N_2O_6$: C, 70.30; H, 5.51; N, 5.47. Found: C, 69.98; H, 5.22; N, 5.94.

2,2'-Anhydro-1-[3-O-(phenoxythiocarbonyl)-5-O-(4-methoxytrityl)-β-D-arabinofuranosyl]uracil (7). To a stirred suspension of compound **5** (11.0 g, 22 mmol) and 4-dimethylaminopyridine (7.3 g, 60 mmol) in dry acetonitrile (600 mL), phenyl chlorothionocarbonate (5.0 g, 29 mmol) was added dropwise under nitrogen. After the reaction mixture was stirred at room temperature for 36 h (monitored by TLC, R_f 0.75, CH_2Cl_2/CH_3OH, 10:1, v/v), the solvent was evaporated in vacuo to dryness yielding a residue which was partitioned between ethyl acetate (250 mL) and water (100 mL). The water phase was extracted with ethyl acetate (2 x 100 mL). The combined ethyl acetate solution was washed with water, dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness under diminished pressure to yield the crude product as a foam (12.6 g, 90%), which was not stable for silica gel column chromatography and therefore used immediately for the next preparation without further purification.

2,2'-Anhydro-1-[3-O-(phenoxythiocarbonyl)-5-O-(4-methoxytrityl)-β-D-arabinofuranosyl)-5-methyluracil (8). This compound was synthesized from compound **6** as described for the synthesis of compound **7**: yield, 4.8 g (92%) and it was also used immediately for the next preparation without further purification.

2,2'-Anhydro-1-[3-deoxy-5-O-(4-methoxytrityl)-β-D-threo-pentofuranosyl]uracil (9). To a stirred suspension of **7** (12.6 g, 20 mmol) and AIBN (2.9 g, 18 mmol) in dry toluene, *n*-Bu_3SnH (10.0 g, 34 mmol) was added dropwise under nitrogen at room temperature. The mixture was stirred at 110-120 °C for 6 h, after which the solvent was evaporated to dryness in vacuo to yield a residue. The residue was then chromatographed on a silica gel (300 g) column (CH_2Cl_2/CH_3OH, 10:1, v/v) to afford 4.2 g (45%) of product as a white foam: TLC, R_f 0.22 (CH_2Cl_2/CH_3OH, 20:1, v/v); NMR ($CDCl_3$) δ 2.20-2.40 (m, 2H, 3'-H), 2.85 (m, 2H, 5'-H), 3.70 (s, 3H, -OCH_3), 4.40-4.55 (m, 1H, 4'-H), 5.30 (m, 1H, 2'-H), 5.80 (d, 1H, 5-H), 5.95 (d, 1H, 1'-H), 6.75-7.35 (m, 15H, ArH and 6-H); UV (CH_3OH) λ_{max} 234 nm (ε 18500), and shoulder at 250 nm (ε 9700). Anal. Calcd. for $C_{29}H_{26}N_2O_5$: C, 72.18; H, 5.43; N, 5.81. Found: C, 71.89; H, 5.30; N, 5.80.

2,2'-Anhydro-1-[3-deoxy-5-O-(4-methoxytrityl)-β-D-threo-pentofuranosyl]-5-methyluracil (10). This compound was synthesized from compound **8** as described for the synthesis of compound **9**: yield, 2.7 g (73%) as a foam; TLC, R_f 0.65 (CH_2Cl_2/CH_3OH, 15:1, v/v); NMR ($CDCl_3$) δ 1.85 (s, 3H, 5-CH_3), 2.20-2.40 (m, 2H, 3'-H), 2.85-2.95 (m, 2H, 5'-H), 3.75 (s, 3H, -OCH_3), 4.45-4.55 (m, 1H, 4'-H), 5.25 (m, 1H, 2'-H), 5.98 (d, 1H, 1'-H), 6.80-7.30 (m, 15H, ArH and 6-H); UV (CH_3OH) λ_{max} 234 nm (ε 22300), and shoulder at 248 nm (ε 13000). Anal. Calcd. for $C_{30}H_{28}N_2O_5$: C, 72.56; H, 5.68; N, 5.64. Found: C, 72.16; H, 5.53; N, 5.90.

1-[3-Deoxy-5-O-(4-methoxytrityl)-β-D-threo-pentofuranosyl]uracil (11). A mixture of **9** (5.1 g, 10.6 mmol), 1 N NaOH (26 mL), and 50% ethanol (380 mL) was stirred at room temperature for 2.5 h. The solution was neutralized with HOAc/EtOH (1:1, v/v) to ~pH 7 and then evaporated in vacuo to yield a white solid, which was collected by filtration, washed with water, dried ($MgSO_4$), and recrystallized from ethanol to give 4.7 g (88%) of product as white crystal: mp 163-165 °C; TLC, R_f 0.44 (CH_2Cl_2/ CH_3OH, 15:1, v/v); NMR ($CDCl_3$) δ 1.80-2.50 (m, 2H, 3'-H), 3.40 (m, 2H, 5'-H), 3.72 (s, 3H, -OCH_3), 4.20 (m, 1H, 4'-H), 4.56 (m, 1H, 2'-H), 5.25 (s, 1H, 2'-OH, D_2O exchangeable), 5.35 (d, 1H, 5-H), 5.95 (s, 1H, 1'-H), 6.80-7.35 (m, 14H, ArH), 7.82 (d, 1H, 6-H), 10.3 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 265 nm (ε 10000), λ_{min} 251 nm. Anal. Calcd. for $C_{29}H_{28}N_2O_6$: C, 69.58; H, 5.64; N, 5.60. Found: C, 69.64; H, 5.73; N, 5.56.

1-[3-Deoxy-5-O-(4-methoxytrityl)-β-D-threo-pentofuranosyl]-5-methyluracil (12). This compound was synthesized from compound **10** as described for the synthesis of compound **11**: yield, 2.1 g (82%) as a white solid; mp 122-124 °C; TLC, R_f 0.50 (CH_2Cl_2/CH_3OH/EtOAc, 10:1:1, v/v); NMR ($CDCl_3$) δ 1.58 (s, 3H, 5-CH_3), 2.25-2.30 (m, 2H, 3'-H), 3.14-3.28 (m, 2H, 5'-H), 3.75 (s, 3H, -OCH_3), 4.11-4.15 (m, 1H, 4'-H), 4.30-4.33 (m, 1H, 2'-H), 5.28 (d, 1H, 2'-OH, D_2O exchangeable), 5.90 (d, 1H, 1'-H), 6.90-7.40 (m, 15H, ArH and 6-H), 11.2 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 268 nm (ε 10300), λ_{min} 252 nm. Anal. Calcd. for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.45. Found: C, 70.20; H, 5.83; N, 5.50.

1-[2,3-Dideoxy-2-methylene-5-O-(4-methoxytrityl)-β-D-glycero-pentofuranosyl]uracil (15). To a stirred suspension of CrO_3 (1.9 g), pyridine (3 mL), and acetic anhydride (1.9 mL) in methylene chloride (10 mL) was added compound **11** (2.8 g, 5.5 mmol) in methylene chloride (10 mL). The reaction mixture was

stirred at room temperature for 45 min. The dark-brown solution was poured into 350 mL of ethyl acetate and the resulting mixture was filtered through a 2 cm-layer of silica gel in a sinter-glass filter. The precipitated solid and silica gel were washed with ethyl acetate (150 mL). The combined filtrate and washings were evaporated in vacuo (< 25 °C) to dryness to give 1-[2,3-dideoxy-2-keto-5-O-(4-methoxytrityl)-β-D-glycero-pentofuranosyl]uracil (**13**), which was used for the next preparation without further purification.

n-Butyllithium (14.5 mL, 1.6 M solution in hexane, 23.0 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (9.9 g, 27.5 mmol) in dry THF (140 mL) at 0 °C (ice-water bath) under nitrogen. The mixture was stirred at 0-5 °C for 10 min and a solution of compound **13** in THF (25 mL) was added dropwise to the ylide solution at 0 °C. The reaction mixture was stirred at room temperature for an additional 4 h and then treated with aqueous ammonium chloride solution (1 M, 45 mL). The solution was extracted with ethyl acetate (2 x 150 mL) and the combined organic phase was washed with water (2 x 50 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated in vacuo to dryness. The residue was dissolved in THF (60 mL) in which 540 mg of NaH (80% in mineral oil, 18 mmol) was added. The mixture was stirred at room temperature for 3 h and treated with aqueous ammonium chloride solution (1 M, 45 mL). The solution was again extracted with ethyl acetate (2 x 150 mL), washed with water (2 x 50 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated in vacuo to a small volume and chromatographed on a silica gel column (CH_2Cl_2/EtOAc, 1;1, v/v, R_f 0.68) to afford 0.74 g of product (27%) as a foam: NMR ($CDCl_3$) δ 2.75 (m, 2H, 3'-H), 3.32 (m, 2H, 5'-H), 3.75 (s, 3H, -OCH_3), 4.25 (m, 1H, 4'-H), 5.24-5.50 (m, 3H, 2'-methylene and 5'-H), 6.50 (s, 1H, 1'-H), 7.10-7.50 (m, 14H, ArH), 7.60 (d, 1H, 6-H), 9.25 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 260 nm (ε 9700), λ_{min} 248 nm. Anal. Calcd. for $C_{30}H_{28}N_2O_5$: C, 72.56; H, 5.68; N, 5.64. Found: C, 72.38; H, 5.64; N, 5.34.

1-[2,3-Dideoxy-2-methylene-5-O-(4-methoxytrityl)-β-D-glycero-pentofuranosyl]-5-methyluracil (16). This compound was synthesized from compound **12** as described for the synthesis of compound **15**: yield, 0.52 g (26%) as a white foam; TLC, R_f 0.69 (CH_2Cl_2/CH_3OH/EtOAc, 10:0.5:0.5, v/v); NMR ($CDCl_3$) δ 1.52 (s, 3H, 5-CH_3), 2.70-2.90 (m, 2H, 3'-H), 3.30-3.50 (m, 2H, 5'-H), 3.78 (s, 3H, -OCH_3), 4.25-4.35 (m, 1H, 4'-H), 5.18 (d, 1H, 2'-methylene-H_A), 5.32 (d, 1 H, 2'-methylene-H_B), 6.62 (s, 1H, 1'-H), 7.25-7.55 (m, 15H, ArH and 6-H), 9.43 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 268 nm (ε 10600), λ_{min} 252 nm. This compound was used for the next preparation without further purification.

2',3'-Dideoxy-2'-methylene-5'-O-4-methoxytritylcytidine (18). To a cooled solution (0 °C, ice-water bath) of compound **15** (0.6 g, 1.2 mmol) and 1,2,4-triazole (1.1 g, 15.6 mmol) in anhydrous pyridine (11 mL) was added dropwise 4-chlorophenyl phosphorodichloridate (0.9 mL, 5.2 mmol) with stirring. After the reaction mixture was stirred at room temperature for 24 h, the solvent was evaporated in vacuo to dryness. The residue (compound **17**) was coevaporated several times with dioxane and then dissolved in a mixture of NH_4OH (16 mL) and dioxane (50 mL). The solution was stirred at room temperature for 2.5 h and the solvent was evaporated to dryness in vacuo. The residue was dissolved in CH_2Cl_2 (100 mL), washed with water (2 x 50 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated in vacuo to a small volume and chromatographed on a silica gel column (CH_2Cl_2/EtOAc/CH_3OH, 10:2:1, v/v) to yield 0.22 g (36%) of compound **18** as a white foam: NMR ($CDCl_3$) δ 2.69 (m, 2H, 3'-H), 3.17 (m, 2H, 5'-H), 3.73 (s, 3H, -OCH_3), 4.21 (m, 1H, 4'-H), 5.00 (d, 1H, 2'-methylene-H_A), 5.20 (d, 1H, 2'-methylene-H_B), 5.57 (d, 1H, 5-H), 6.49 (s, 1H, 1'-H), 7.15-7.18 (d, 2H, 4-NH_2, D_2O exchangeable), 7.22-7.39 (m, 14H, ArH), 7.49 (d, 1H, 6-H); UV (CH_3OH) λ_{max} 274 nm (ε 8700), λ_{min} 260 nm. Anal. Calcd. for $C_{30}H_{29}N_3O_4$: C, 72.70; H, 5.90; N, 8.48. Found: C, 72.39; H, 5.77; N, 8.63.

2',3'-Dideoxy-2'-methylenecytidine (19) and Its Hydrochloride Salt (20). A suspension of compound **18** (0.4 g, 0.8 mmol) in 80% acetic acid (8.5 mL) was stirred at room temperature until the reaction was completed (~3.5 h, monitored by TLC). The resulting solution was evaporated under reduced pressure to dryness and then coevaporated with CH_2Cl_2 (2 x 5 mL). The residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 7:1, v/v, R_f 0.23) to afford 0.13 g (72%) of product as a white foam: MS *m/z* 224 (M+1), 113 [$C_6H_9O_2$ (sugar residue)], 112 (cytosine +1); NMR (Me_2SO-d_6) δ 2.61 (m, 2H, 3'-H), 3.50 (m, 1H, 5'-H_A), 3.60 (m, 1H, 5'-H_B), 4.04 (m, 1H, 4'-H), 4.91 [t (overlap dd), 1H, 5'-OH, D_2O exchangeable], 4.95 (dd, 1H, 2'-methylene-H_A, J = 2.2 Hz), 5.16 (dd, 1H, 2'-methylene-H_B, J = 2.2 Hz), 5.71 (d, 1H, 5-H, J = 7.4 Hz), 6.43 (s, 1H, 1'-H), 7.15-7.21 (d, 2H, 4-NH_2, D_2O exchangeable), 7.53 (d, 1H, 6-H, J = 7.4 Hz).

Compound **19** (0.12 g) was converted to its hydrochloride salt **20** by treatment with acetyl chloride (0.35 mg, 4.3 mmol) in methanol (8 mL), followed by evaporation in vacuo to dryness. The resulting solid was recrystallized from methanol to afford 0.1 g (70%) of product as white crystals: mp 200 °C (dec.); NMR (Me_2SO-d_6) δ 2.67 (m, 2H, 3'-H), 3.35 (br s, 1H, 5'-OH, D_2O exchangeable), 3.50 (m, 1H, 5'-H_A), 3.60 (m, 1H, 5'-H_B), 4.15 (m, 1H, 4'-H), 5.18 (d, 1H, 2'-methylene-H_A, J = 1.7 Hz), 5.28 (t, 1H,

2'-methylene-H_B, J = 1.9 and 2.0 Hz), 6.13 (d, 1H, 5-H, J = 7.8 Hz), 6.36 (s, 1H, 1'-H), 7.98 (d, 1H, 6-H, J = 7.8 Hz), 8.78 (s, 1H, 4-NH_A, D_2O exchangeable), 9.84 (s, 1H, 4-NH_B, D_2O exchangeable); UV (CH_3OH) λ_{max} 279 nm (ε 8000), λ_{min} 247 nm; (0.01 N HCl) λ_{max} 278 nm (ε 10600), λ_{min} 242 nm; (0.01 N NaOH) λ_{max} 270 nm (ε 7000), λ_{min} 250 nm. Anal. Calcd. for $C_{10}H_{14}ClN_3O_3$: C, 46.25; H, 5.43; N, 16.18. Found: C, 46.54; H, 5.03; N, 15.92.

2',3'-Dideoxy-2'-methyleneuridine (21). A suspension of compound **15** (0.31 g, 0.62 mmol) in 80% acetic acid (5 mL) was stirred at room temperature. A clear solution was obtained after 30 min and the reaction was completed 1 h later (monitored by TLC). The reaction mixture was evaporated under reduced pressure to dryness. The residue was coevaporated with ethanol (2 x 5 mL) and was purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 10:1, v/v, R_f 0.45) to afford 0.1 g (72%) of product as a glass: MS *m/z* 225 (M^+-1), 113 [$C_6H_9O_2$ (sugar residue) and uracil+1], 112 (uracil); NMR (Me_2SO-d_6) δ 2.65 (m, 2H, 3'-H), 3.48 (m, 1H, 5'-H_A), 3.58 (m, 1H, 5'-H_B), 4.07 (m, 1H, 4'-H), 4.92 (t, 1H, 5'-OH, D_2O exchangeable), 5.05 (dd, 1H, 2'-methylene-H_A, J = 2.3 and 2.4 Hz), 5.24 (dd, 1H, 2'-methylene-H_B, J = 2.2 Hz), 5.61 (d, 1H, 5-H, J = 8.1 Hz), 6.36 (s, 1H, 1'-H), 7.57 (d, 1H, 6-H, J = 8.1 Hz), 11.3 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 262 nm (ε 9800), λ_{min} 232 nm; (0.01 N HCl) λ_{max} 262 nm (ε 11900), λ_{min} 232 nm; UV (0.01 N NaOH) λ_{max} 262 nm (ε 10700), λ_{min} 234 nm. Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.56; H, 5.40; N, 12.50. Found: C, 53.85; H, 5.45; N, 12.27.

2',3'-Dideoxy-2'-methylene-5-methyluridine (22). A mixture of compound **16** (0.52 g, 1.0 mmol) and 80% acetic acid (5 mL) was stirred at room temperature for 1.5 h. The reaction mixture was evaporated under reduced pressure to dryness. The residue was coevaporated with methylene chloride (4 x 5 mL) and purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 10:1, v/v, R_f 0.48) to afford a solid, which was recrystallized from ethyl acetate to yield 0.12 g (74%) of product as white crystals: mp 132-134 °C; MS *m/z* 239 (M^++1), 127 (thymine + 1), 113 [$C_6H_9O_2$ (sugar residue)]; NMR (Me_2SO-d_6) δ 1.74 (s, 3H, 5-CH_3), 2.66 (m, 2H, 3'-H), 3.52 (m, 1H, 5'-H_A), 3.59 (m, 1H, 5'-H_B), 4.07 (m, 1H, 4'-H), 4.92 (t, 1H, 5'-OH, D_2O exchangeable), 5.02 (d, 1H, 2'-methylene-H_A, J = 2.0 Hz), 5.23 (d, 1H, 2'-methylene-H_B, J = 2.0 Hz), 6.35 (s, 1H, 1'-H), 7.40 (s, 1H, 6-H), 11.3 (s, 1H, 3-NH, D_2O exchangeable); UV (CH_3OH) λ_{max} 267 nm (ε 9700), λ_{min} 235 nm; UV (0.01 N HCl) λ_{max} 267 nm (ε 9700), λ_{min} 236 nm; UV (0.01 N NaOH) λ_{max} 267 nm

(ε 8800), λ_{min} 237 nm. Anal. Calcd. for $C_{11}H_{14}N_2O_4$: C, 55.45; H, 5.92; N, 11.76. Found: C, 55.84; H, 5.93; N, 11.52.

Cytotoxicity Test Procedures in Vitro. Cytotoxicity 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 their exponential phase of growth. The cell number of the drug-free cultures (control), as well as that of the cultures supplemented with the test compounds, was determined after 24, 48, and 72 h of growth.

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(22) 2',3'-Dideoxy-2'-methylene-5'-Q-trityluridine (as foam): TLC, R_f 0.66 ($CHCl_3$/EtOAc, 1:1, v/v); NMR (500 Hz, $CDCl_3$) δ 2.71 (d, 2H, 3'-H), 3.12 (m, 1H, 5'-H_A), 3.22 (m, 1H, 5'-H_B), 4.26 (m, 1H, 4'-H), 5.11 (d, 1H, 2'-methylene-H_A, J = 1.8 Hz), 5.29 (d, 1H, 2'-methylene-H_B, J = 2.0 Hz), 5.41 (d, 1H, 5-H, J = 8.0 Hz), 6.39 (s, 1H, 1'-H), 7.25-7.38 (m, 15H, ArH), 7.49 (d, 1H, 6-H, J = 8.0 Hz), 11.4 (d, 1H, 3-NH, D_2O exchangeable). Anal. Calcd. for $C_{29}H_{26}N_2O_4 \cdot 0.25H_2O$: C, 73.94; H, 5.67; N, 5.95. Found: C, 73.90; H, 5.81; N, 5.87.

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