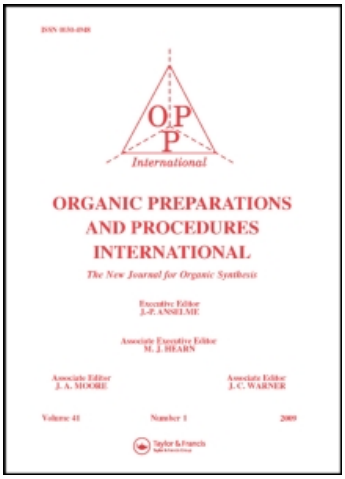


Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

SYNTHESIS OF 2',3'-DIDEOXY-5-METHYLCYTIDIN-2-ENE, A CYTIDINE ANALOG OF 3'-DEOXYTHYMIDIN-2'-ENE (d4T)

Tai-Shun Lin[a]; Jing-Hua Yang[a]
[a] Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT

To cite this Article Lin, Tai-Shun and Yang, Jing-Hua(1990) 'SYNTHESIS OF 2',3'-DIDEOXY-5-METHYLCYTIDIN-2-ENE, A CYTIDINE ANALOG OF 3'-DEOXYTHYMIDIN-2'-ENE (d4T)', Organic Preparations and Procedures International, 22: 2, 265 — 268
To link to this Article: DOI: 10.1080/00304949009458203
URL: http://dx.doi.org/10.1080/00304949009458203

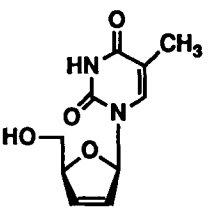
ORGANIC PREPARATIONS AND PROCEDURES INT. 22(2), 265-268 (1990)

SYNTHESIS OF 2',3'-DIDEOXY-5-METHYLCYTIDIN-2'-ENE, A CYTIDINE ANALOG OF 3'-DEOXYTHYMIDIN-2'-ENE (d4T)

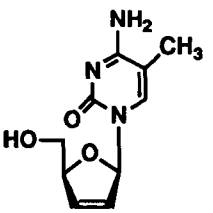
Tai-Shun Lin* and Jing-Hua Yang

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

3'-Deoxythymidin-2'-ene (2',3'-dideoxy-2',3'-didehydrothymidine, d4T), a thymidine analog, first synthesized by Horwitz et al.,[1] was recently reported to have potent in vitro anti-HIV-1 activity in several cell lines.[2-7] The in vitro toxicity of d4T against normal human hematopoietic progenitor cells (CFU-GM) was measured in comparison to AZT, which is currently the only FDA approved drug against AIDS. d4T reduces colony-forming units by 50% at a concentration of 100 μM (ID_{50} = 100 μM), whereas AZT is 100 times more toxic, with an ID_{50} value of 1 μM.[7] Based on these findings, 2',3'-dideoxy-5-methylcytidin-2'-ene (1, 5-Me-d4C), a cytidine analog of d4T, was synthesized via two pathways as a potential anti-HIV agent.

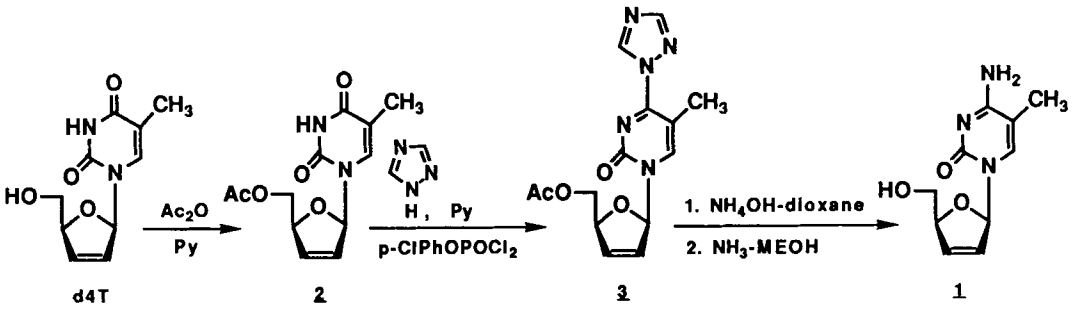


d4T

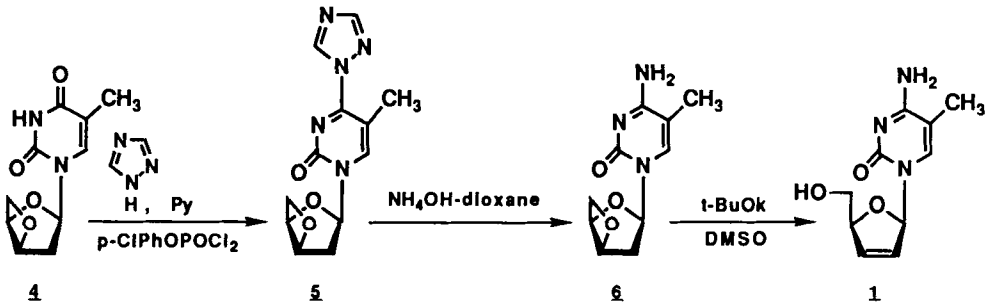


1 (5-Me-d4C)

The synthesis of choice utilized the key starting material, d4T, which was prepared by the method of Horwitz et al.[1] from thymidine. Acetylation of d4T with acetic anhydride in pyridine gave the acetate 2. Reaction[8,9] of 2 with 1,2,4-triazole and 4-chlorophenyl phosphorodichloridate in dry pyridine at room temperature yielded the 4-triazolylpyrimidinone derivative 3, which was then treated with NH_4OH-dioxane, followed by methanolic ammonia to afford the desired 2',3'-dideoxy-5-methylcytidin-2'-ene (1) as shown below.



An alternative synthesis of 1 involved treatment of 3',5'-epoxythymidine (4)[1] with 1,2,4-triazole and 4-chlorophenyl phosphorodichloridate in dry pyridine to produce the 4-triazolylpyrimidinone 5. Ammoniolysis of 5 with NH_4OH-dioxane yielded the 4-amino derivative 6. Treatment[10] of 6 with potassium t-butoxide in DMSO afforded the final product 1.



EXPERIMENTAL SECTION

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. Thin layer chromatography was performed using EM silica gel 60 F_{254} sheets (0.2 mm). The UV spectra were recorded using a Beckman 25 spectrophotometer. The NMR spectra were obtained on a Bruker WM 500 spectrometer at 500 MHz using Me_4Si as an internal reference and the mass spectra (at 70 eV) were provided by Yale University Chemical Instrumentation Center. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT.

3'-Deoxy-5'-O-acetylthymidin-2'-ene (2).- Acetic anhydride (2.5 mL) was slowly added to a stirred solution of 3'-deoxythymidin-2'-ene (d4T, 0.67 g, 3 mmol) in 30 mL of dried pyridine at 0° (ice-water bath). The solution was allowed to stand overnight at 4°. The solvent and the excess acetic anhydride were removed in vacuo to give a residue, which was then triturated with water. The resultant white solid precipitate was collected, washed with water and dried. The crude product (0.72 g, 90%) was then recrystallized from ethanol, mp. 187-188°; UV (MeOH): λ_{max} 265nm (ε 9760), λ_{min} 234nm; UV (0.01 N NaOH): λ_{max} 266nm (ε 8163), λ_{min} 244nm; UV (0.01 N HCl): λ_{max} 266nm (ε 9760), λ_{min} 236nm; MS: m/e 267 (M^+ + 1), 127 (thymine +

1); NMR (Me_2SO-d_6): δ 1.80 (s, 3H, 5-CH_3), 2.05 (s, 3H, 5'-OAc), 4.24 (d, 2H, 5'-H), 4.95 (m, 1H, 4'-H), 5.95 (dd, 1H, 2'-H, vinyl), 6.35 (dd, 1H, 3'-H, vinyl), 6.78 (d, 1H, 1'-H), 7.22 (s, 1H, 6-H).

Anal. Calcd for $C_{12}H_{14}O_5N_2$: C, 54.14; H, 5.26; N, 10.53
Found: C, 53.79; H, 5.36; N, 10.46

2',3'-Dideoxy-5-methylcytidin-2'-ene (1). Method A.- 4-Chlorophenyl phosphorodichloridate (0.71 mL, 4.36 mmol) was added dropwise to a solution of compound 2 (0.58 g, 2.18 mmol) and 1,2,4-triazole (0.60 g, 8.72 mmol) in dry pyridine (25 mL) at 0° (ice-water bath). The reaction mixture was stirred at room temperature for 3.5 days after which the solvent was removed in vacuo at ~ 30°. The resulting residue (compound 3) was dissolved in 60 mL of NH_4OH-dioxane (1:3, v/v) and stirred in a Wheaton pressure bottle for 4 hrs at 0°. This solvent was then removed under reduced pressure. The remaining dark-colored residue was redissolved in saturated methanolic ammonia (60 mL) and stirred overnight in a Wheaton pressure bottle at 4°. The solution was evaporated to dryness in vacuo. The residue was dissolved in methanol and clarified with Norit. The solvent was removed again and the resulting reddish-yellow oil was chromatographed on a silica gel (100 g) column (CH_2Cl_2-MeOH, 4:1, v/v) to yield 0.13 g (26%) of product as a glass. UV (MeOH): λ_{max} 279nm (ε 5809), λ_{min} 239nm; UV (0.01 N NaOH): λ_{max} 278nm (ε 6145), λ_{min} 237nm; UV (0.01 N HCl): λ_{max} 285nm (ε 8546), λ_{min} 245nm; MS: m/e 224 (M^+ + 1), 126 (5-methylcytosine + 1); NMR (Me_2SOd_6): δ 1.78 (s, 3H, 5-CH_3), 3.67 (m, 2H, 5'-H), 4.73 (dd, 1H, 4'-H), 5.00 (t, 1H, 5'-OH, D_2O exchangeable), 5.85 (m, 1H, 3'-H, vinyl), 6.32 (m, 1H, 2'-H, vinyl), 6.87 (br s, 1H, 4-NH_a, D_2O exchangeable), 6.89 (d, 1H, 1'-H), 7.28 (br s, 1H, 4-NH_b, D_2O exchangeable), 7.53 (s, 1H, 6-H).

Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.81; H, 5.83; N, 18.83
Found: C, 53.54; H, 6.17; N, 18.56

2'-Deoxy-3',5'-epoxy-5-methylcytidine (6).- To a stirred suspension of the cyclic ether 4 (0.96 g, 4.28 mmol) and 1,2,4-triazole (1.38 g, 20 mmol) in 50 mL of dried pyridine, 4-chlorophenyl phosphorodichloridate (1.63 mL, 10.0 mmol) was added dropwise at 0° (ice-water bath). The reaction mixture was stirred at room temperature for 70 hrs. The solvent was removed in vacuo to produce a reddish-black residue (compound 5), which was then dissolved in 80 mL of NH_4OH-dioxane (1:3, v/v) and stirred in a Wheaton pressure bottle at room temperature for 5 hrs. The solution was clarified with Norit and filtered. The filtrate was evaporated to dryness under diminished pressure. The resulting residue was chromatographed on a silica gel (100 g) column (CH_2Cl_2-MeOH, 4:1, v/v) to afford 0.37 g (39%) of product which was then recrystallized from acetone, mp. 178-181°; UV (MeOH): λ_{max} 280nm (ε 4515), λ_{min} 262nm; UV (0.01 N NaOH): λ_{max} 278nm (ε 5000), λ_{min} 256nm; UV (0.01 N HCl): λ_{max} 287nm (ε 7573), λ_{min} 246nm; MS: m/e 224 (M^+ + 1), 126 (5 methylcytosine + 1); NMR (Me_2SOd_6): δ 1.84 (s, 3H, 5-CH_3), 2.29-2.36 (m, 1H, 2'-H_a), 2.40-2.43 (m, 1H, 2'-H_b), 4.04 (dd, 1H,

5'-H_a), 4.65 (dd, 1H, 5'-H_b), 4.87 (m, 1H, 4'-H), 5.43 (t, 1H, 3'-H), 6.50 (dd, 1H, 1'-H), 6.85 (br s, 1H, 4-NH_a, D_2O exchangeable), 7.35 (br s, 1H, 4-NH_b, D_2O exchangeable), 7.90 (s, 1H, 6-H).

Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.81; H, 5.83; N, 18.83
Found: C, 53.52; H, 6.16; N, 18.53

2',3'-Dideoxy-5-methylcytidin-2'-ene (1). Method B.- Compound 6 (0.19 g, 0.85 mmol) and potassium t-butoxide (0.15 g, 1.30 mmol) were dissolved in dry DMSO (7 mL). The reaction mixture was stirred at room temperature for 70 hrs. Water (0.5 mL) and MeOH (2 mL) were added to the solution and then neutralized with HOAc-EtOH (1:1, v/v) to ~ pH 7.5. The solvents were evaporated to dryness in vacuo at 60-65°. The remaining residue was chromatographed on a preparative TLC plate (Anatech Unitplate, 2 mm) using CH_2Cl_2-MeOH (1:1, v/v) as the eluent to afford a glassy product (0.06 g, 32%). The UV, MS and NMR spectroscopic data of this compound were identical to those data obtained for compound 1 in Method A as previously described.

Acknowledgment.- This research was supported by PHS Grant CA-45410 awarded by the National Cancer Institute, DHHS. We also acknowledge the support of Northeast NMR Facility at Yale University for the high resolution NMR spectra, made possible by a grant from the Chemical Division of the National Science Foundation (CHE-7916210).

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(Received September 25, 1989; in revised form December 22, 1989)