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Synthesis of Some ^{3}H Labeled 2',3'-Dideoxynucleosides of Pharmacological Interest

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SUMMARY

[Pyrimidine-5-^{3}H]2',3'-dideoxycytidine and [ribose 2,3-^{3}H]2',3'-dideoxyinosine are prepared by catalytic reduction with tritium gas. [Adenine-8-^{3}H]2'3'-dideoxyadenosine is prepared by catalytic exchange with tritium gas. [Hypoxanthine-8-^{3}H]2',3'-dideoxyinosine is prepared by the action of adenosine deaminase on [adenine-8-^{3}H]2',3'-dideoxyadenosine.

Key words: 2',3'-dideoxycytidine, 2',3'-dideoxyinosine, 2',3'-dideoxyadenosine, tritium, NSC-606170, NSC-98700, NSC-612049

INTRODUCTION

Among the compounds investigated for activity against the HIV-1 virus associated with acquired immune deficiency syndrome, (AIDS), some of the 2',3'-dideoxynucleosides have shown promise.[1] To expedite pharmacological studies on these compounds, we have prepared 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine, and 2',3'dideoxycytidine labeled with tritium in the base-moiety and 2',3'-dideoxyinosine labeled with tritium in the carbohydrate residue.

Results and Discussion

2',3'-Dideoxy-5-bromocytidine (1) was prepared by adapting the procedure of Matsuda *et al.*[2] for the bromination of cytidine. Compound 1 was catalytically dehalogenated with carrier-free tritium gas using palladium on carbon catalyst in tetrahydrofuran-water as shown in Chart 1 to give [pyrimidine-5-^{3}H]2',3'-dideoxycytidine (2). Compound 2 was back-exchanged with methanol and

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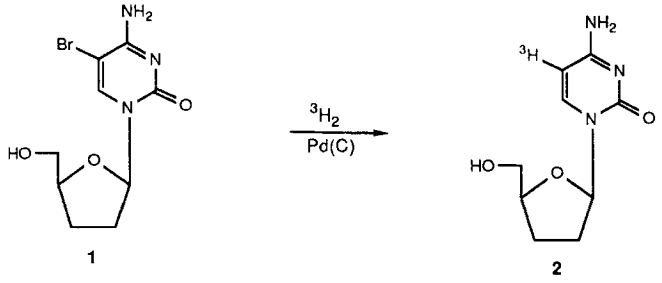
purified by preparative TLC and HPLC. The specific activity, determined by UV absorption, was 25.2 Ci/mmol.

Chart 1

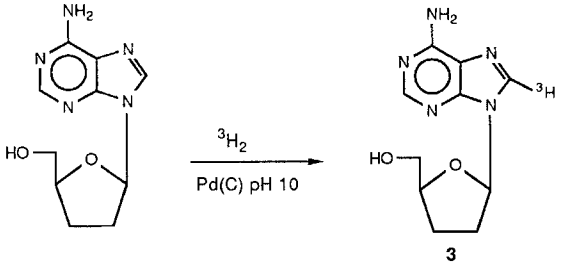


1 2

Because of the resistance of adenine to bromination, [adenine-8-^{3}H]2',3'-dideoxyadenosine (3) was prepared by an exchange procedure similar to that reported for adenosine by Evans et al.[3] Tritium gas and palladium on carbon catalyst in a pH 10 carbonate-bicarbonate buffer was used, as shown in Chart 2A. Back-exchange with methanol and purification by preparative-TLC gave 3 with a specific activity of 2.65 Ci/mmol. Subjection of 3 to the action of adenosine deaminase in pH 7.5 phosphate buffer at room temperature gave [hypoxanthine-8-^{3}H]2',3'-dideoxyinosine (4), as shown in Chart 2B. The salts and enzyme could be separated from 4 by chromatography on C_{18}-bonded silica. Reductive tritiation of the unsaturated analogue (5)[4] afforded [ribose-2,3-^{3}H]2',3'-dideoxyinosine (6) (see Chart 2C). A deuterium experiment had shown by mass spectrum ten to fifteen percent exchange into the hypoxanthine residue of 6, but this was found to be readily removed by catalytic back-exchange using palladium on carbon in methanol. The product was purified by preparative-TLC to give 6 with specific activity of 31.6 Ci/mmol.

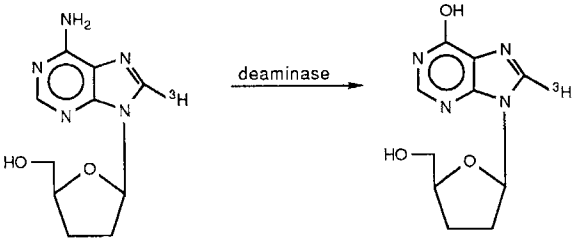
Each of the tritiated nucleosides was stirred for 24 h at room temperature (21°C) in pH 7 phosphate buffer, and the water was then lyophilized and analyzed for exchangeable tritium. All the nucleosides showed less than one percent exchangeable tritium under these conditions. Radiochemical purity was established using radio-TLC and radio-HPLC. Samples were hydrolyzed with dilute hydrochloric acid, and the products were analyzed by radio-TLC to determine the specificity of the location of the tritium. None of the base-

Chart 2A



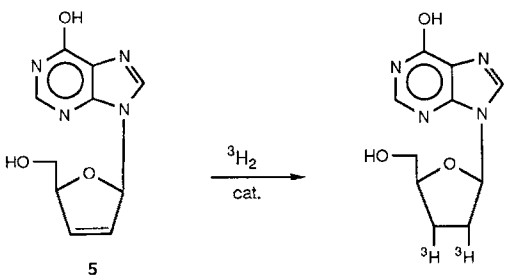
3

Chart 2B



3 4

Chart 2C



5 6

labeled nucleosides had more than two percent of the tritium in the sugar moiety. The [ribose-2,3-^3H]2',3'-dideoxyinosine had less than two percent of the tritium on the hypoxanthine moiety. ^3H NMR confirmed these results. The spectrum of compound 2 showed the expected doublet at δ 5.7 (J = 7 Hz) for C_5-H, which collapsed to a singlet at δ 5.66 when proton-decoupled. Compound 6 gave complex multiplets at δ 2.42 for C_2'-H and δ 2.07 for C_3'-H, corresponding closely to previously reported values for 2',3'-dideoxyadenosine.[5] These collapsed to broad singlets at δ 1.96 and δ 2.39 when proton-decoupled. Com-

pound 3 gave the expected singlet at δ 8.32 for C_8-H. No other tritium signals were observed, indicating that at least 95% of the label was at the expected locations.

EXPERIMENTAL

Ultraviolet spectra were recorded on a Varian Model 2290 spectrometer. Mass spectra were performed on an AEI-MS902 high resolution mass spectrometer. ^{3}H NMR spectra were recorded on a JEOL FX90Q Fourier transform spectrometer. E. Merck silica gel 60 F-254 and Analtech cellulose and DEAE-cellulose analytical plates were used for analytical TLC. Radioactive samples were counted on a Packard Tri-Carb 4000 liquid scintillation spectrometer using internal standard in Scintiverse E cocktail. Developed TLC plates were scanned on a Berthold Model LB 283 Linear Analyzer system. HPLC was done using a Waters Assoc. Model 6000A dual pump system with a Model U6K septum-less injector and a Berthold Model LB503-HDS radioactivity monitor as detector. Carrier-free tritium gas was purchased from DuPont/New England Nuclear Corporation. Samples of the unlabeled nucleosides were provided by the National Cancer Institute.

2',3'-Dideoxy-5-bromocytidine (1). A solution of 84 mg, (0.4 mmol) of 2',3'-dideoxycytidine in 2 mL of pyridine and 2 mL of acetic acid was treated with a solution of 63 mg (0.4 mmol) of bromine in 0.34 mL of acetic acid. The mixture was stirred at room temperature for 18 h. The volatiles were removed under reduced pressure. The residue was twice dissolved in ethanol and evaporated, after which the residue was dissolved in methanol and treated with excess strong-base ion exchange resin (Bio-Rad AG-1X8) in the hydroxide form. The resin was filtered from the solution, and the solvent was evaporated to give the crude product 1. The product was chromatographed on silica gel TLC eluted with chloroform-methanol-diethylamine (65:30:5). The desired band was washed from the silica gel with chloroform-ethanol (1:1) to give 1 as a clear gum. The product was crystallized from methanol to yield 23 mg of white crystals: mp 162.5-163°C (dec). The TLC (silica gel, chloroform-methanol 7:3 R_f 0.86) showed no impurity. Spectral data were consistent with the structure: IR(KBr) 3460, 3340, 3050, 2920, 1645, 1635 cm^{-1}; NMR (CD_3OD) δ

8.64(s); 5.98, 5.97, 5.95, 5.94 (dd); 4.17 (m); 3.94, 3.69 (double ABq J = 3.2 Hz and 12 Hz); 2.47 (m); 2.06 (m); 1.92 (m); mass spectrum m/z 289, 291, 276, 274, 272, 270, 189 (100), 191.

Anal. Calcd for $C_9H_{12}BrN_3O_3$: C, 37.26; H, 4.17; Br, 27.55; N, 14.48. Found: C, 37.24; H, 4.23; Br, 27.69; N, 14.37.

[Pyrimidine-5-^{3}H]2',3'-Dideoxycytidine (2). A solution of 6.8 mg (0.023 mmol) of 2',3'-dideoxy-5-bromocytidine, 1, in 0.5 mL of tetrahydrofuran-water (9:1) with 5 mg of 5% palladium on carbon was exposed to 5 curies of carrier-free tritium gas at room temperature for 3 h. The reaction mixture was filtered, and the solvent was evaporated under vacuum. The residue was twice dissolved in methanol and evaporated under vacuum.

The crude product was chromatographed on HPLC using a Waters Assoc. 10 µm C_{18} Radial-Pak column eluted with 20% methanol in 0.2 M ammonium acetate, flow rate 1 mL/min. The solvent was removed from the product fractions, and the residue was chromatographed on two 20 x 20 cm x 0.25 mm silica gel 60 F plates eluted with ethyl acetate-methanol-water (3:2:1). The product bands were washed from the silica with methanol to give 208 mCi of 2 that was at least 98% pure by radio-TLC [SiO_2, ethyl acetate-methanol-H_2O (4:2:1) R_f 0.45 and chloroform-methanol-diethylamine (60:35:5) R_f 0.5] and by radio-HPLC [Altex Ultrasphere ODS 5µm, linear gradient from 0.05 M ammonium acetate to 70% acetonitrile in 0.05 M ammonium acetate in 30 min, 1 mL/min t_R 13 min]. The specific activity based on UV absorbance [λ_{max} (methanol) 273 nm, ε 7987) was 119 mCi/mg or 25.2 Ci/mmol. Exchange in pH 7 phosphate buffer for 24 h at room temperature showed less than 1% exchangeable tritium. Acid hydrolysis showed 99% of the total tritium in the pyrimidine residue. Proton decoupled ^3H NMR (DMSO-d_6) δ 5.66 (s, 5-^{3}H) corresponds to δ 5.70 (d, J = 7 Hz) for the unlabeled material.

[Adenine-8-^{3}H]2',3'-Dideoxyadenosine (3). A mixture of 10 mg (0.0425 mmol) of 2',3'-dideoxyadenosine and 5 mg 10% palladium on carbon in 0.3 mL of pH 10 sodium carbonate-sodium bicarbonate buffer was exposed to 5 Ci of carrier-free tritium gas for 4.5 h. The catalyst was removed by filtration, and the water was removed by freeze-drying. The residue was dissolved

in methanol and passed through a column of ~ 1 cc silica gel. The methanol eluant was removed under vacuum. The product was exchanged with methanol 4 more times and chromatographed on a 20 x 20 cm x 0.25 mm silica gel plate eluted with chloroform-methanol-diethylamine (60:35:5). The product band was washed from the silica with methanol to give 78.8 mCi of product that was pure by radio-TLC [SiO_2, $CHCl_3$-MeOH-Et_2NH (60:35:5) R_f, 0.58; and ethyl acetate-methanol (1:1) R_f 0.40; and cellulose; water, R_f 0.73] and radio-HPLC [Altex Ultrasphere-ODS 5 μm, 25 cm; 5% methanol in 0.01 M KH_2PO_4 pH 5.6 to 60% methanol, linear gradient in 30 min, 2 mL/min, t_R 13.8 min]. The specific activity was determined to be 2.65 Ci/mmol (11.2 mCi/mg) by UV absorbance λ_{max} (methanol) 260 ε 15,900. Back-exchange in pH 7 phosphate buffer indicated less than 1% exchange in 24 h at room temperature. Acid hydrolysis showed 98% of the total tritium in the purine residue. Tritium NMR (DMSO-d_6) δ 8.32 (s, 8-^{3}H) corresponds to δ 8.36 (s) for the unlabeled material.

[Hypoxanthine 8-^{3}H]2',3'-Dideoxyinosine (4). A solution of 55 mCi (5 mg, 0.02 mmol) of [purine-8-^{3}H]2',3'-dideoxyadenosine in 1 mL of 0.1 N Na_2HPO_4 adjusted to pH 7.5 with NaOH was treated with 1 mg (39 units) of adenosine deaminase type VII at room temperature (~ 21°C) for 2 h. The reaction mixture was passed through a column of 1.2 g C_{18}-reverse phase bonded silica, and the column was eluted with 5 mL H_2O. The product was washed from the column with 10 mL methanol-H_2O (2:1). The yield of 52.7 mCi (95%) of [hypoxanthine-8-^{3}H]2',3'-dideoxyinosine showed no 2',3'-dideoxyadenosine and was 98% pure by radio-TLC [silica gel, chloroform-methanol-diethylamine (60:35:5), R_f 0.54; DEAE-cellulose, H_2O, R_f 0.78; and cellulose, H_2O, R_f 0.73] and radio-HPLC [Altex Ultrasphere-ODS, 5 μ, 25 cm, 5% methanol in 0.01 M KH_2PO_4 pH 5.6 to 60% methanol, linear gradient in 30 min, 1 mL/min, t_R 16 min]. The specific activity was determined by UV λ_{max} (MeOH) 250 ε = 12,200 to be 2.61 Ci/mol (11.03 mCi/mg), the same as the starting material. Back-exchange in pH 7 phosphate buffer at room temperature showed less than 1% exchange in 24 h. Acid hydrolysis showed 99% of the total tritium in the hypoxanthine residue.

[Ribose-2,3-^{3}H]2',3'-Dideoxyinosine (6). A solution of 8 mg (0.034 mmol) of the unsaturated analogue (5)[4] in 1 mL of DMF with 5 mg of 5% palladium on

carbon was exposed to 5 Ci of carrier-free tritium gas for 2 h at ambient temperature. The catalyst was removed by filtration; the solvent was removed under reduced pressure, and the residue was back-exchanged twice with methanol. The crude product was dissolved in 10 mL of methanol, and 1.5 mg of 10% palladium on carbon was added, and the mixture was stirred for 19 h at ambient temperature. The mixture was filtered through a 0.5 μm PTFE filter, evaporated, and exchanged twice more with methanol. The product was chromatographed on one 20 x 20 cm x 0.25 mm silica gel plate eluted with $CHCl_3$-methanol-diethylamine (60:35:5). The product band was washed from the silica with methanol. Compound 6 showed no impurity by radio-TLC [DEAE-cellulose, H_2O, R_f 0.80 and cellulose, H_2O, R_f 0.81] or radio-HPLC [Altex Ultrasphere-ODS 5 μm, 250 mm x 4.6 mm; 5% methanol in 0.01 M KH_2PO_4, pH 5.6 to 80% methanol, linear gradient in 30 min, 1 mL/min, t_R 12.6 min]. The UV-spectrum corresponded to that reported[6] for inosine λ_{max} (MeOH) 251 nm. The specific activity, determined by UV using ε 12,200[6], was 31.6 Ci/mmol (134 mCi/mg). The yield was 152 mCi. Back-exchange in pH 7 phosphate buffer for 24 h at room temperature showed 0.2% exchangeable tritium. Acid hydrolysis showed >98% of the total tritium in the dideoxyribose residue. Proton decoupled ^3H NMR (DMSO-d_6) δ 1.96 (bs, 3'-^3H) and 2.39 (bs, 2'-^3H)) correspond to δ 2.05 (m) and 2.40 (m) for the unlabeled material.

Hydrolysis Procedures. Approximately 1 mCi of each tritiated nucleoside was diluted with 10 mg of unlabeled material and dissolved in 0.5 mL dilute HCl. The inosine nucleosides were completely hydrolyzed in 1 h in 0.1 N HCl at room temperature (~ 21°C). 2',3'-Dideoxyadenosine required 24 h in 0.1 N HCl at room temperature, and 2',3'-dideoxycytosine required 4 h in 1 N HCl at 100°C. The purine nucleoside hydrolysates were analyzed by radio-TLC on DEAE-cellulose-F (H_2O). The pyrimidine nucleoside hydrolysate was analyzed on cellulose-F (n-butanol-methanol-H_2O-conc. NH_4OH 60:20:20:1). The base fragments were visualized by UV light, and the deoxyribose residues by V_2O_5-H_2SO_4 spray reagent.[7]

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