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SYNTHESIS OF TRITIUM-LABELLED 5-CHLORO-2',3'-DIDEOXY-3'-FLUOROURIDINE (935U83) - A SELECTIVE ANTI-HIV AGENT

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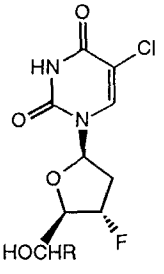
SUMMARY

[5'-^{3}H]-5-Chloro-2',3'-dideoxy-3'-fluorouridine (1; R=[^{3}H]) was prepared at a specific activity of 10.2 Ci/mmol suitable for development of a radioimmunoassay procedure. The synthetic sequence employed controlled oxidation of unlabelled 1 to the 5'-aldehyde (2), isolation as the imidazolidine adduct (3), regeneration of the free aldehyde, reduction with [^{3}H]$NaBH_4$, and purification by preparative TLC. The radiochemical purity was 98.0%.

Key Words: 5-chloro-2',3'-dideoxy-3'-fluorouridine, 935U83, anti-HIV agent, tritium labelling, radioimmunoassay.

INTRODUCTION

5-Chloro-2',3'-dideoxy-3'-fluorouridine (935U83, 1; R=H) is a selective anti-HIV (human immunodeficiency virus) nucleoside analogue that has shown activity against HIV strains that are resistant to AZT, ddI, or ddC (1).



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935U83 is less toxic *in vitro* and *in vivo* than AZT, and is currently undergoing phase I/II clinical trials.

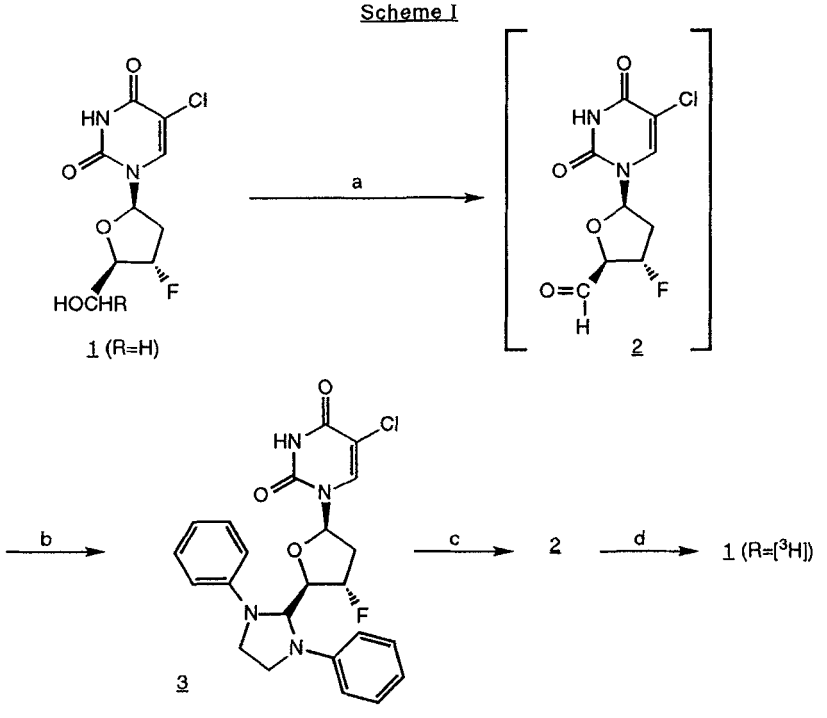
Development of a radioimmunoassay procedure for analysis of clinical trial samples required a high specific activity tritium-labelled form of 935U83. The studies demanded that the radiolabelled 935U83 should be chemically stable, should have a high chemical and radiochemical purity, and a specific activity of 10-12 Ci/mmol. This paper describes the preparation of tritium-labelled 935U83 with a specific activity of 10.2 Ci/mmol by a short oxidation-reduction sequence from unlabelled 935U83.

RESULTS AND DISCUSSION

Methods for the synthesis of unlabelled 1 have been described by Burns et al. (2), and Selway et al. (3).

For the synthesis of [^{3}H]1, we chose a synthetic sequence (shown in Scheme I) involving controlled oxidation of unlabelled 1 to the 5'-aldehyde 2, isolation of 2 as the imidazolidine adduct 3, regeneration of 2, and reduction with [^{3}H]$NaBH_4$. The route is a combination of methodology successfully used in the earlier syntheses of tritiated antiviral compounds zidovudine (4) and desciclovir (5).

Scheme I



a) Pyridinium trifluoroacetate, DMSO, DCC; $(CO_2H)_2$
b) 1,2-Dianilinoethane
c) THF, Amberlite IR-118H resin
d) [^{3}H]$NaBH_4$, 2-propanol/H_2O/NaOH

Oxidation of unlabelled 1 with dry DMSO and 1,3-dicyclohexylcarbodiimide in the presence of pyridinium trifluoroacetate (6) was shown to be complete after 3 h at 25°C. After treatment with oxalic acid, the reaction mixture was filtered to remove 1,3-dicyclohexylurea, and the aldehyde solution was treated with Wanzlick's reagent, 1,2-dianilinoethane (7). The resulting solid was isolated and purified by chromatography on silica gel to give a 39.4% yield of the adduct (2*R*, 4*S*, 5*R*)-5-chloro-1-[5-(1,3-diphenyl-2-imidazolidinyl)-4-fluoro-tetrahydro-2-furyl]uracil (3).

Regeneration of the free 5'-aldehyde 2 was effected by treatment of 3 in THF solution with an aqueous suspension of the acidic resin Amberlite IR-118H. The aldehydic proton appeared at δ 9.45 in the ^{1}H NMR spectrum. Due to its instability, the crude aldehyde was not fully characterized, and was used rapidly in the borohydride reaction without further purification.

Reduction of 60 μM of the crude aldehyde 2 with 30 μM of $NaBH_4$ in 2-propanol/H_2O/0.1*N* NaOH solution gave a white residue containing crude 1 (69.5% by HPLC). Three successive preparative TLC purifications on SiO_2 using a) CH_2Cl_2:MeOH (19:1), b) CH_2Cl_2:EtOAc (1:1), and then c) EtOAc, afforded 3.2 mg of white crystalline 1 in 20.4% yield from 2. The product was shown to be of excellent purity by TLC (single-spot), HPLC (97.8%), and by ^{1}H NMR.

The radiolabelled synthesis of [^{3}H] 1 was carried out using essentially the same reaction conditions as described above, and the details are included in the experimental section. Three successive PTLC purifications resulted in the isolation of 8.1 mg (97.1% by HPLC) of white crystalline [^{3}H] 1 in 51.3% yield from 2. Dilution with an appropriate weight of unlabelled 1 resulted in the isolation of [^{3}H] 1 with a specific activity of 10.2 Ci/mmol. The radiolabelled material was shown to co-chromatograph with an authentic sample of 1 by HPLC and TLC. The radiochemical purity was 99.3% by HPLC radiodetection and 98.0% by radioactive plate-scanning.

The [^{3}H] 1 has been used successfully (8) in the development of a radioimmunoassay system for 935U83.

EXPERIMENTAL

Sodium boro[^{3}H]hydride was obtained from Amersham Corporation at a batch specific activity of 67.3 Ci/mmol. Unlabelled 5-chloro-2',3'-dideoxy-3'-fluorouridine (935U83, 1) was obtained from Burroughs Wellcome Co., Chemical Development Laboratories. 1,3-Dicyclohexylcarbodiimide, pyridinium trifluoroacetate and 1,2-dianilinoethane were purchased from Aldrich Chemical Company. Amberlite IR-118H acidic resin was purchased from Sigma Chemical Company. All other solvents and reagents were of reagent

purity and were obtained from readily available commercial sources. Tetrahydrofuran and dimethyl sulphoxide were dried over Type 4Å Molecular Sieves.

High pressure liquid chromatography (HPLC) was performed using a Waters 510 Pump, a Waters Lambda-Max Model 481 LC Spectrophotometer, a Hewlett-Packard 3392A Integrator, and the following conditions - Beckman Ultrasphere C_{18} 5μ 4.6 mm x 25 cm column, mobile phase MeCN/H_2O/$HClO_4$ (350/650/0.1, v/v), and flow rate 0.7 mL/min with UV detection at 275 nm. Radiochemical purity of the final product was measured using a Beckman 171 Radioisotope Detector interfaced with the HPLC system and Ready Flow II (Beckman) liquid scintillation cocktail. Analytical thin layer chromatography (TLC) was performed on 5 x 20 cm glass plates pre-coated with 0.25 mm silica gel 60 (E. Merck) using EtOAc as mobile phase. Preparative thin layer chromatography (PTLC) was performed on 20 x 20 cm glass plates pre-coated with 2 mm silica gel 60 F_{254}s with concentration zone (E. Merck). Column chromatography was performed on silica gel 60 (70-230 mesh; E. Merck). Proton NMR spectra were obtained in DMSO-d_6 using a Varian XL-300 spectrometer (300 MHz). Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Radiochemical purity was determined by radiochromatogram scanning of a TLC plate using a Bioscan System 200 Imaging Scanner. Mass spectral analysis was performed by Oneida Research Services, Inc., Whitesboro, N.Y. on a Finnegan 4500 mass spectrometer using methane chemical ionization. The specific activity was determined by counting an aliquot of a solution whose concentration had been determined by UV spectroscopy using a Beckman 6000 liquid scintillation counter and Ready Safe (Beckman) liquid scintillation cocktail.

(2*R*,4*S*,5*R*)-5-Chloro-1-[5-(1,3-diphenyl-2-imidazolidinyl)-4-fluoro-tetrahydro-2-furyl]-uracil (3)

A stirred solution of 5-chloro-2',3'-dideoxy-3'-fluorouridine (1) (500 mg; 1.89 mmol) and pyridinium trifluoroacetate (183 mg; 0.948 mmol; 0.5 eq.) in dry DMSO (5.0 mL) at 25°C under nitrogen was treated with 1,3-dicyclohexylcarbodiimide (DCC) (1.17 g; 5.67 mmol; 3.0 eq.), and the suspension was stirred for 3 h. At this time, TLC showed the complete disappearance of 1. The reaction was quenched by the gradual addition of anhydrous oxalic acid (340 mg; 3.78 mmol; 2.0 eq.) and, after stirring for 7 min, the mixture was filtered and the white solid 1,3-dicyclohexylurea was washed with acetonitrile (3 x 5 mL). The clear amber *filtrate* was treated with 1,2-dianilinoethane (401 mg; 1.89 mmol; 1.0 eq.) and the solution was stirred under *nitrogen for 90 min. The solution was poured into water* (25 mL) and the mixture was extracted with EtOAc (2 x 25 mL). The organic phase was evaporated to dryness under reduced pressure and dried *in vacuo* at 42°C overnight to give a yellow solid.

This material was chromatographed on a silica gel column (150 mL, equilibrated in CH_2Cl_2) using an elution gradient ranging from EtOAc/CH_2Cl_2 (2/98) to EtOAc/CH_2Cl_2 (5/95). The relevant fractions were

combined and the solvents removed under high vacuum to give (2*R*, 4*S*, 5*R*)-5-chloro-1-[5-(1,3-diphenyl-2-imidazolidinyl)-4-fluoro-tetrahydro-2-furyl]uracil (3) (340 mg; 39.4%) as a white solid foam; m.p. 142-145°C (dec.); TLC: single-spot material R_f = 0.65; ^{1}H NMR: δ 2.33-2.49 (m, 2 H, 2'-H), 3.61-3.71 (br m, 4 H, N-CH_2CH_2-N), 4.36 and 4.51 (2 br d, 1 H, 4'-H), 5.30 and 5.60 (2 m, 1 H, 3'-H), 5.93 (d, 1 H, 5'-H), 6.12 (t, 1 H, 1'-H), 6.71-6.98 (m, 6 H, 2-, 4- and 6-Ar-H), 7.24 (dd, 4 H, 3- and 5-Ar-H), 7.77 (s, 1 H, 6-H), 11.95 (br s, 1 H, NH); MS (CI + CH_4): *m/e* (relative intensity) 457 (20.1, $[M+H]^+$), 437 (11.5, $[M-F]^+$), 311 (100, [M-5-chlorouracil]$^+$), 291 (70.6, [M-HF-5-chlorouracil]$^+$), 223 (69.7, [*N,N'*-diphenylimidazolidinyl]$^+$), 147 (7.3, [5-chlorouracil + 2H]$^+$). Anal. Calcd. for $C_{23}H_{22}ClFN_4O_3$•0.25EtOAc: C, 60.19; H, 5.05; N, 11.70; Cl, 7.40%. Found: C, 60.12; H, 5.16; N, 11.49; Cl, 7.60%.

5-Chloro-1-(2,3-dideoxy-3-fluoro-β-*D-erythro*-pentodialdofuranosyl)uracil (2)

A solution of the adduct 3 (870 mg) in THF (40 mL) was added to a mixture of Amberlite IR-118H acidic resin (16 g wet, pre-washed with H_2O until pH ~7, then with THF) in H_2O (24 mL), and the resulting mixture was stirred at room temperature under nitrogen. After 3 h, TLC showed a small amount of residual 3, so further resin (4 g wet) was added and stirring continued. After a further 1 h, TLC still showed a trace of residual 3, and the pH of the mixture had dropped to ~5. The mixture was filtered and the resin was washed with THF (3 x 20 mL). The pale-yellow filtrate was evaporated to dryness *in vacuo* to give the crude 5'-aldehyde 2 (444.7 mg; 93.2%) as a pale-yellow solid; TLC: virtually single-spot material R_f = 0.50; ^{1}H NMR: δ 2.35-2.48 (m, 2 H, 2'-H), 3.96 and 4.10 (dd, 1 H, 4'-H), 5.25 and 5.52 (2 m, 1 H, 3'-H), 6.22 (dd, 1 H, 1'-H), 8.27 (s, 1 H, 6-H), 9.45 (s, 1 H, 5'-H), 11.95 (br s, 1 H, NH).

5-Chloro-2',3'-dideoxy-3'-fluorouridine (1)

The procedure for preparation of 1 from 2 was essentially identical to that used below in the preparation of [^{3}H] 1. From 15.6 mg of 2 and 1.1 mg of $NaBH_4$ was obtained 3.2 mg (20.4%) of white solid 1; TLC: single-spot material R_f = 0.45; HPLC: 97.8% (t_R = 4.54 min); ^{1}H NMR: δ 2.39-2.48 (m, 2 H, 2'-H), 3.62-3.68 (m, 2 H, 5'-H), 4.15 and 4.28 (dt, 1 H, 4'-H), 5.19 and 5.46 (2 m, 1 H, 3'-H), 5.34 (t, 1 H, -OH), 6.20 (dd, 1 H, 1'-H), 8.29 (s, 1 H, 6-H), 11.94 (br s, 1 H, NH).

[5'-^{3}H]-5-Chloro-2',3'-dideoxy-3'-fluorouridine (1)

A mixture of crude aldehyde 2 (15.6 mg; 60 μM) in 2-propanol (3.0 mL) was cooled to 0-5°C and was added rapidly to a stirred mixture of sodium boro[^{3}H]hydride (1.26 mg; 30 μM; 2.0 Ci at ~67.3 Ci/mmol) in 2-propanol (2.0 mL), H_2O (0.5 mL) and 0.1*N* NaOH solution (6 drops) at 0-5°C under argon. The cooling-bath was removed and the mixture stirred at 25°C for 60 min. Acetone (0.5 mL) was added and the mixture

was stirred for a further 10 min, then cooled to 0-5°C and treated dropwise with 0.1*N* HCl solution until pH ~7. After evaporation to dryness under reduced pressure, the white solid residue was redissolved in EtOH (2.0 mL) - HPLC showed 64.2% 1 (t_R = 4.56 min). The ethanolic solution was applied to a PTLC plate, and the plate developed in CH_2Cl_2:MeOH (19:1). The band corresponding to authentic 1 was removed, extracted with acetone (25 mL), the mixture filtered and the solution concentrated to about 2 mL - HPLC showed 75.1% 1 (t_R = 4.44 min). The acetone solution was applied to a second PTLC plate, and the plate developed in CH_2Cl_2:EtOAc (1:1). The band corresponding to authentic 1 was removed, extracted with acetone (25 mL), the mixture filtered and the solution concentrated to about 2 mL - HPLC showed 96.8% 1. The solution was applied to a third PTLC plate, and the plate developed in EtOAc. The band corresponding to authentic 1 was removed, extracted with acetone (25 mL), and the mixture filtered through Celite 545 to give a clear solution - HPLC showed 97.1% 1. The solution was evaporated to dryness *in vacuo* to give the [^{3}H] 1 (8.1 mg; 51.3%) as a white crystalline solid.

The appropriate amount (3.6 mg) of unlabelled 1 was added to decrease the specific activity to the desired 10-12 Ci/mmol range, and the solids were dissolved in EtOAc (3.0 mL).

TLC: showed single-spot material with R_f = 0.44 corresponding to authentic 1. One minor impurity was detected by radioactive scanning of the TLC plate, and the radiochemical purity was 98.0%.

HPLC: showed 98.2% (t_R = 4.37 min) as compared to 99.3% (t_R = 4.36 min) for authentic 1. Radiodetection gave a value for the radiochemical purity of 99.3%.

The final yield of white crystalline solid [^{3}H] 1 was 11.7 mg (449.3 mCi) with specific activity 10.2 Ci/mmol.

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