AN IMPROVED SYNTHESIS OF 5-FLUORO-2'-DEOXYURIDINE INCORPORATING ISOTOPIC LABELS

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SUMMARY

The title compound has been synthesized by a new route that requires less steps and gives higher overall yields than existing procedures. The synthesis makes use of low molecular weight starting materials that are commercially available in isotopically enriched forms and thereby enables the specific labelling of carbons in the pyrimidine ring. An improved synthesis of the versatile reagent methyl propiolate is also included.

Key Words: Methyl Propiolate, Fluorination, 5-Fluoro-2'-deoxyuridine, Pyrimidine Labelled Synthesis, Nucleoside

INTRODUCTION

With the advent of pulsed Fourier transform nmr it is now possible to examine nuclei routinely even when they are present in very low concentration. In particular, this new ability enables one to observe directly the individual nuclei of proteins or of substrates bound to an enzyme, thereby enhancing the scope of nmr as a useful tool in biochemistry. A recent example from our laboratories demonstrated the applicability of ^{19}F NMR for this purpose; we were able to gain valuable information concerning the mode of binding of the inhibitor, 5-fluoro-2'-deoxyuridylate, in its covalent ternary complex with thymidylate synthetase and 5,10-methylenetetrahydrofolate (1). 5-Fluorouracil, which is the precursor of the latter inhibitor, is commonly employed in the treatment of skin and gastrointestinal cancers and in recently developed combination chemotherapeutic regimens for certain cancers. The inhibition of thymidylate synthetase by 5-fluoro-2'-deoxyuridylate represents the primary effective mode of action of the drug, 5-fluorouracil. Still more information is to be gleaned from ^{13}C NMR

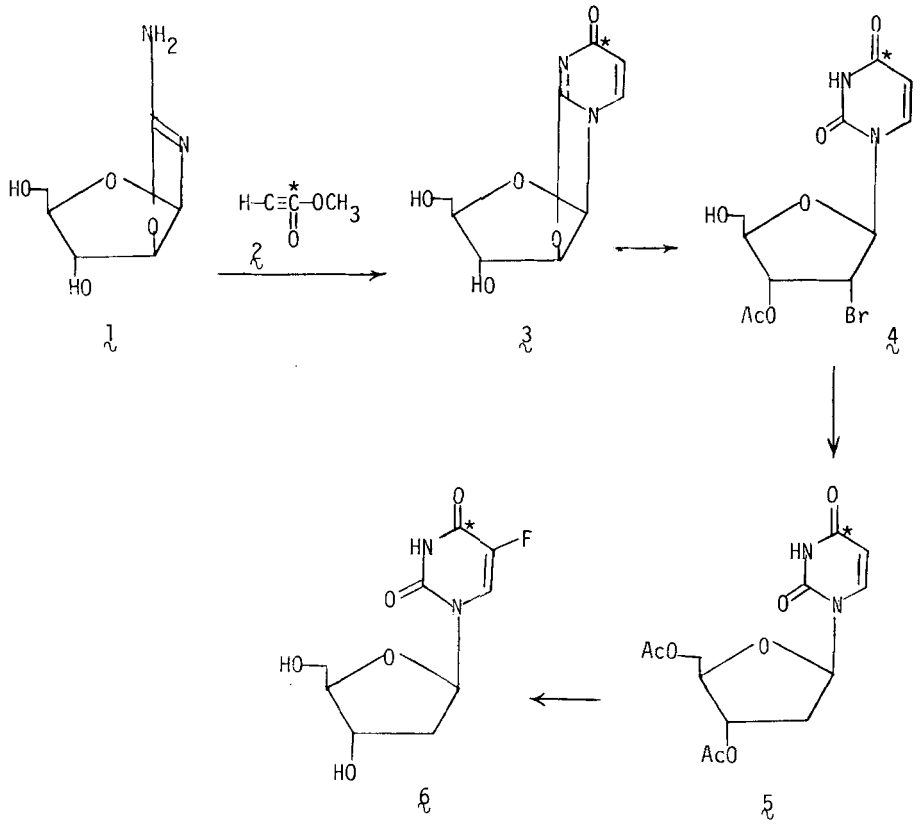
0362-4803/79/0216-0335$01.00

Received June 23, 1978
Revised July 17, 1978

spectra of this complex, but the low natural abundance of the carbon-13 isotope dictates the need to increase artificially its isotopic content in order to improve the sensitivity of the NMR experiment. To accomplish this we have designed an efficient synthesis of 5-fluoro-2'-deoxyuridine (FdU) that enables specific carbons in the pyrimidine ring to be isotopically enriched.

The synthesis outlined in the Scheme proceeds with a minimum of steps and with an overall yield of FdU of 24% from labeled barium carbonate, or 41.4% from 1. Although designed with the special intention of labelling for NMR purposes, this Scheme represents a very efficient preparation of FdU competitive with existing methods (2-3), both in terms of yield and simplicity. In particular, it obviates the need to separate α and β anomers late in the synthesis and takes advantage of some recent developments in nucleoside chemistry.

SCHEME



The condensation of methyl propiolate 2 with the oxazoline 1 was based on a route successfully applied by Holý to prepare $O^{2,2'}$-anhydro-L-uridine (4) and the α-anomer of 3 (5,6), although we found that a large excess of ester was unnecessary. Cleavage of the 2,2'-anhydro bond in 3 was readily achieved with acetyl bromide in the presence of base, as shown by Honjo et al. (7). The 2'-bromine was subsequently removed by the action of tributyltin hydride and a radical initiator (6). The fluorination of the blocked nucleoside 5 using CF_3OF according to Naik and Robins (8,9) proceeded without complication, although their procedure was modified slightly to improve the yields. Alternatively, as shown by Cech et al. (10,11), 5 can be directly fluorinated with F_2.

An initial difficulty encountered was the formation of methyl propiolate, for which no truly efficient preparation existed (12). The most common preparation of propiolate acid, carbonylation of sodium acetylide, was deemed unsuitable for a labeling synthesis in view of the excess of acetylene and high pressure of carbon dioxide required (13-15). Small scale (80 mmol) pilot experiments verified the capricious nature of this method and prompted our investigation of an alternative synthesis. Both ethynyl magnesium bromide (16) and lithium acetylide were found to be preferable to sodium acetylide because of their ease of formation and rapid, almost quantitative, uptake of CO_2. Both of these reagents gave comparable and reproducible yields in terms of ^{13}C-labeled CO_2. However, since the lithium acetylide can be formed quantitatively from acetylene, it was the reagent employed when ^{13}C-labeled acetylene was used to label the 5 and 6 positions of the uracil ring. Both methods are included in the Experimental.

Diazomethane was used to esterify the propiolic acid because of complications encountered in the normal acid-catalyzed alcohol condensation (17). An excess of diazomethane must be avoided because of its further reaction with the ester to form a pyridazole[1] (18,19). Isolation of the propiolate ester by fractional distillation resulted in greatly reduced yields. Since this purification was

[1]Addition of a large excess of diazomethane in ether at room temperature leads to the quantitative formation of 1-methyl-3-carboxymethylpyridazole.

found to be unnecessary, the ester was used directly after concentrating the solution in which it was prepared to a small volume.

In conjunction with other proton NMR experiments aimed at elucidating the relative orientation of the pyrimidine and ribose and portions of FdU, we also selectively deuterated the 6-, 1'- and 2'-positions in the molecule. The 6-hydrogen is readily exchanged for deuterium by a base (NaOD) catalyzed exchange at 55°C in D_2O, essentially as described by Cushley et al. (20), followed by treatment with Dowex 50 (H^+) ion exchange resin and lyophilization. The 1'-deutero FdU was prepared by using the appropriately deuterated arabinose-1-d starting material (21, 22). The 2'-position was deuterated by employing tributyltin deuteride (prepared using lithium aluminum tetradeuteride) in the debromination step. It may also be noted that the 2-carbon and either nitrogen could easily have been labeled via this Scheme by using the appropriately labeled cyanamide (23).

EXPERIMENTAL SECTION

Melting points are uncorrected and were taken in open capillary tubes. Nuclear magnetic resonance spectral data are reported in ppm (δ) deshielded with respect to tetramethylsilane; the abbreviations used for the coupling constants (Hz) are: d(doublet) and q(quartet). Only significant absorptions are given for structural assignment. Ultraviolet spectra (UV) were recorded on a Beckman Acta V spectrophotometer. Barium carbonate (90% ^{13}C) was obtained from Koch Isotopes, acetylene (90% ^{13}C) from Merck, and CF_3OF from PCR Inc. Other reagents were obtained from commercial sources.

Methyl propiolate-1-^{13}C (2). Method A.[2] A solution of ethyl magnesium bromide was prepared from 1.8 g of magnesium and 8.9 g of ethyl bromide in 50 mL of tetrahydrofuran (THF) in the usual manner. This solution was added dropwise to a saturated solution of acetylene in 100 mL of the THF at room temperature through which acetylene was continuously bubbled.[3] Meanwhile, the carbon dioxide

[2]Caution. Propiolic acid is a corrosive liquid and a strong lachrymator, as are its esters.

[3]Commercial acetylene was purified by passage through conc. sulfuric acid, solid potassium hydroxide and activated alumina scrubbers.

prepared from 12.02 g of barium carbonate-^{13}C (24) was transferred, using standard vacuum line techniques, into a 250 ml flask containing 25 mL of the THF frozen at -196°. After warming to -78° and admitting dry nitrogen, this flask was connected to the ethynyl magnesium bromide solution via a cannula. The latter solution was cooled to 10° and transferred dropwise to the (-78°) solution of carbon dioxide. The thick white suspension that resulted was warmed to 25° and stirred an additional hour. Extraction (3 x 60 mL) with saturated, aqueous, acidified ammonium sulfate solution (the addition of up to 200 mL of ether helped break up any emulsions that formed), drying over anhydrous sodium sulfate, and careful fractionation of the solvents gave 3.29 g, (77% from barium carbonate) of propiolate acid-1-^{13}C as a concentrated (50% by weight) solution in THF: ^{1}H NMR (THF) δ 3.2 (d, J_{CH} = 4.8), ^{13}C NMR (THF) δ 153.7 (d, J_{CH} = 4.8). Addition of an equimolar quantity of diazomethane in ether to an ice-cold ether solution of the acid, followed by fractionation of the solvents, gave 3.04 g, (75% from the acid) of methyl propiolate-1-^{13}C: ^{1}H NMR(THF) δ 3.5 (d, J_{CH} = 4) and 3.2 (d, J_{CH} = 5); ^{13}C NMR(THF) δ 153.3 (dq, J_{CH} = 4.7 and 4.7).

Method B. Acetylene (1.14 g, 44 mmol) was condensed into a 250 mL flask containing 100 mL of dry THF frozen at -196° using conventional vacuum line techniques. The flask was warmed to -78° and dry nitrogen was admitted. n-Butyl lithium (40 mL of a 1.1 M solution in hexane) was added over a period of 15 min. The resulting solution was then transferred via cannula using nitrogen pressure to a -50° solution containing 4 mmol of carbon dioxide-^{13}C prepared in 50 mL THF as above. The thick white suspension that resulted was worked up as above to give 2.1 g (68%) of propiolic acid-1-^{13}C.

2,2'-Anhydro-β-D-arabinofuranosyl uracil-4-^{13}C (3). A solution of methyl propiolate-1-^{13}C in 15 mL of THF, containing 6.7 g (80 mmol) of the ester, was added to 13.9 g (80 mmol) of 2-amino-β-D-arabinofurano [1',2':4,5] 2-oxazoline (1) (25) in 70 mL of water and 80 mL of ethanol, and the mixture was refluxed for 5 h. After reducing the volume and cooling, 3 precipitated out as colorless crystals, which were collected on a filter and washed briefly with ice-cold ethanol: 10.7 g

(47%): mp 246-248° [lit. (26) 244-7°]. Concentration of the mother liquor and cooling afforded an additional 3.2 g of product, bringing the total yield to 61.5%: ^{1}H NMR(D_2O) δ 5.67(d, J_{HH} = 8,H_5), and 7.85(dd, J_{HH} = 8, J_{HC} = 8.8, H_6); ^{13}C NMR(D_2O) δ 160.2(d, J_{CH} = 8.8); UV $\lambda_{max}^{H_2O}$ 250 (8000) [lit. (27) 250 (7860)].

2'Bromo-2',5'-di-O-acyl-2'-deoxyuridine-4-^{13}C (4). Compound 3 (5.65 g, 25 mmol) was refluxed with acetyl bromide (9.23 g, 75 mmol) in 18 mL of dimethylformamide and 120 mL of ethyl acetate for 1.5 h. The cooled mixture was washed with water (3 x 50 mL), then dried over anhydrous sodium sulfate. Rotary evaporation of the solvent left a pale brown syrup which was coevaporated with three small portions of ethyl acetate to give an amorphous solid. Yield 9.10 g, 93.5%: mp 55-7° [lit. (7) 67-76°]; UV λ_{max}^{EtOH} 256 (9300) [lit. (12) 256 (10,000)]; ^{1}H NMR($CDCl_3$) δ 5.80(d, J_{HH} = 8,H_5) and 7.7(dd, J_{HH} = 8,J_{HC} = 11.H_6); NMR($CDCl_3$) δ 163.2(d,J_{CH} = 11).

3',5'-Di-O-acyl-2'-deoxyuridine-4-^{13}C (5). To a solution of 4 (4.5 g, 12 mmol) in 50 mL of dry benzene was added 10.0 g (35 mmol) of tri-n-butyltin hydride (100 mL of a 10% solution in benzene[4]) and 65 mg of azo-bis isobutyronitrile. The mixture was refluxed for one hour and then reduced in volume on a rotary evaporator to 50 mL. Addition of excess hexane and cooling induced the precipitation of a viscous oil. This oil was triturated repeatedly with hexane until the odour to tri-n-butyl tin compounds was no longer detectable, whereupon the oil crystallized. Recrystallization from ethanol afforded 2.72 g (83%) of 5 as colorless crystals: mp 108-9° [lit. (29) 107-110°]: UV λ_{max}^{EtOH} 262 (ε = 5600); ^{1}H NMR($CDCl_3$) δ 5.90(d, J_{HH} = 8,H_5) and 7.87(dd, J_{HH} = 8,J_{CH} = 11,H_6); ^{13}C NMR ($CDCl_3$) δ 163.4-(d, J_{CH} = 11).

5-Fluoro-2'-deoxyuridine-4-^{13}C (6). The diacetate 5 (043 g, 1.39 mmol) in 15 mL of chloroform was fluorinated at -78° with 0.30 g (2.9 mmol) of CF_3OF dissolved

[4]Tri-n-butyltin hydride was prepared from tri-n-butyltin chloride and lithium aluminum hydride in ether (28). The distilled product contained as much as 15% unreacted chloride, which was very difficult to remove by distillation. Its presence did not affect the yield of the debromination reaction and the crude product mixture was used in the preparation of 5.

in 10 mL of $CFCl_3$[5]. After one hour at this temperature, excess CF_3OF was removed in a stream of nitrogen. The flask was covered with aluminum foil, and the solvents were removed at 9° using a water aspirator. The flocculent white solid so obtained was treated for 16 h at 0° with 20 mL of a 10% solution of triethylamine in 50% aqueous methanol. This mixture was then applied to a short (0.5 x 10 cm) column of Dowex 50(H^+) and eluted with methanol. The fractions with significant absorption at 268 nm were pooled and evaporated to dryness. Recrystallization from ethanol afforded 0.25 g (86%) of FdU-4-^{13}C: mp and mmp 148-150° [lit. (8,9) 149-150°]; ^1H NMR(D_2O) δ 8.31(dd, J_{HF} = 6.5, J_{HC} - 6.5, H_6); ^{13}C NMR(D_2O) δ 161.9(dd, J_{CF} = 25, J_{CH} = 6.5); UV $\lambda_{max}^{0.1NHCl}$ 268(8500) [lit. (8,9) 268(8400)].

Phosphorylation of this material with $POCl_3$ in pyridine afforded FdUMP which inhibited thymidylate synthetase to an extent quantitatively equivalent to a commercial sample (33).

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[5]Caution. Trifluoromethylhypofluorite has been reported to react violently with certain organic compounds, and is undoubtedly very toxic (30-32). In this work the reagent was transferred from the commercial cylinder, using a Teflon^R tube, to an all-glass calibrated vacuum line which was fitted with Young^R stopcocks lubricated with halocarbon grease. The exact quantity of gas was measured using a narrow bore mercury manometer (the reaction with the mercury is minimal during the short time required for this step). The gas was immediately condensed into the $CFCl_3$ in the reaction vessel, which consisted of two bulbs (one containing 5 in $CHCl_3$, the other $CFCl_3$ frozen at -196°C) connected by a Teflon stopcock. The entire apparatus was equilibrated at -78° using dry ice, whereupon the bulbs were connected and the reaction initiated by tilting the vessel to mix the solutions. Excess CF_3OF was vented off in a hood. Over twenty reactions, using up to 5 g CF_3OF at a time, have been conducted in this manner without incident.

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ACKNOWLEDGEMENTS

The authors acknowledge the contribution of Dr. T. C. Chiu during the initial stages of this work, and helpful comments of Drs. M. J. Robins and R. L. Cargill. The generous gifts of CF_3OF from Drs. W. B. Fox and R. A. DeMarco (Office of Naval Research, Washington) and seed crystals of D-arabinolactone from Dr. H. S. Isbell (American University, Washington) are greatly appreciated. This research was supported by the National Institutes of Health through grants CA15645 and CA12842 from the National Cancer Institute. R.B.D. is the recipient of a Faculty Research Award (FRA-144) from the American Cancer Society.