SYNTHESIS OF 5-DEUTEROMETHYL-2'-DEOXYURIDINE (THYMIDINE-α,α,α-d_3) AND RELATED COMPOUNDS

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SUMMARY

Thymine-α,α,α-d_3 (4) was synthesized from perdeuterated methyl iodide and pyrimidine-lithium. Thymidine-α,α,α-d_3 (8) was then synthesized by coupling compound 4 with 1α-chloro-2-deoxy-3,5-bis(p-toluoyl)-α-D-ribofuranosyl chloride in the presence of Friedal-Crafts catalyst. Thymidine-α,α,α-d_3 (8), a metabolic precursor of deoxyribonucleic acid, has potential application for study of human tissue growth kinetics *in vivo* using a sensitive deuterium micromapping technique.

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INTRODUCTION

Thymidine is a specific metabolic precursor of deoxyribonucleic acid (DNA). If thymidine is present in the extracellular environment of dividing cells, it is incorporated into DNA through the thymidine kinase, or "salvage", enzyme pathway. The thymine moiety of DNA is also synthesized de novo by methylation of deoxyuridine monophosphate through the thymidylate synthetase enzyme pathway.

Radioactive thymidine such as tritiated thymidine has been used experimentally in an autoradiographic technique to identify the type and number of dividing cells in tissues and cell cultures (1). Tritiated thymidine has also been used in the clinical investigation of the kinetics of proliferation of human tissue (2). Unfortunately, there is a potential genetic hazard associated with tritiated thymidine in children and young adults (3). On the other hand, the effective cross section for detection of deuterium by the $D(T,n)^4He$ nuclear reaction is relatively large (about $10^{-24} cm^2$) (4). Recent work on the structural integrity of cells during triton bombardment indicates that the triton fluence could attain about $4 \times 10^{16} cm^{-2}$ before cell nuclei disintegrate (5). If 10% of thymine moieties in DNA in a cell nucleus can be labeled with fully-deuterated 5-methyl group, the nucleus will be labeled with about 5×10^8 excess deuterium atoms. Following maximum triton bombardment these should yield $(5 \times 10^8) \cdot (4 \times 10^{16}) \cdot 10^{-24} = 20$ alpha tracks in a tight radical cluster detectable in the presence of randomly-oriented background tracks. It is estimated that prolonged ($\sim$ several hours) intravenous infusion of deuterated thymidine into a mammal at the rate of about 20 μg/kg/min (6) would be required to attain more than 10% labeling of DNA by exogenous thymidine in dividing cells via the pyrimidine deoxyribonucleoside salvage pathway (7), a condition which should be readily achieved for human clinical studies of cell proliferation. Therefore, we have synthesized thymidine-α,α,α-d_3 (8) and explored the possibility of using it for clinical investigations in children and young adults.

RESULTS AND DISCUSSION

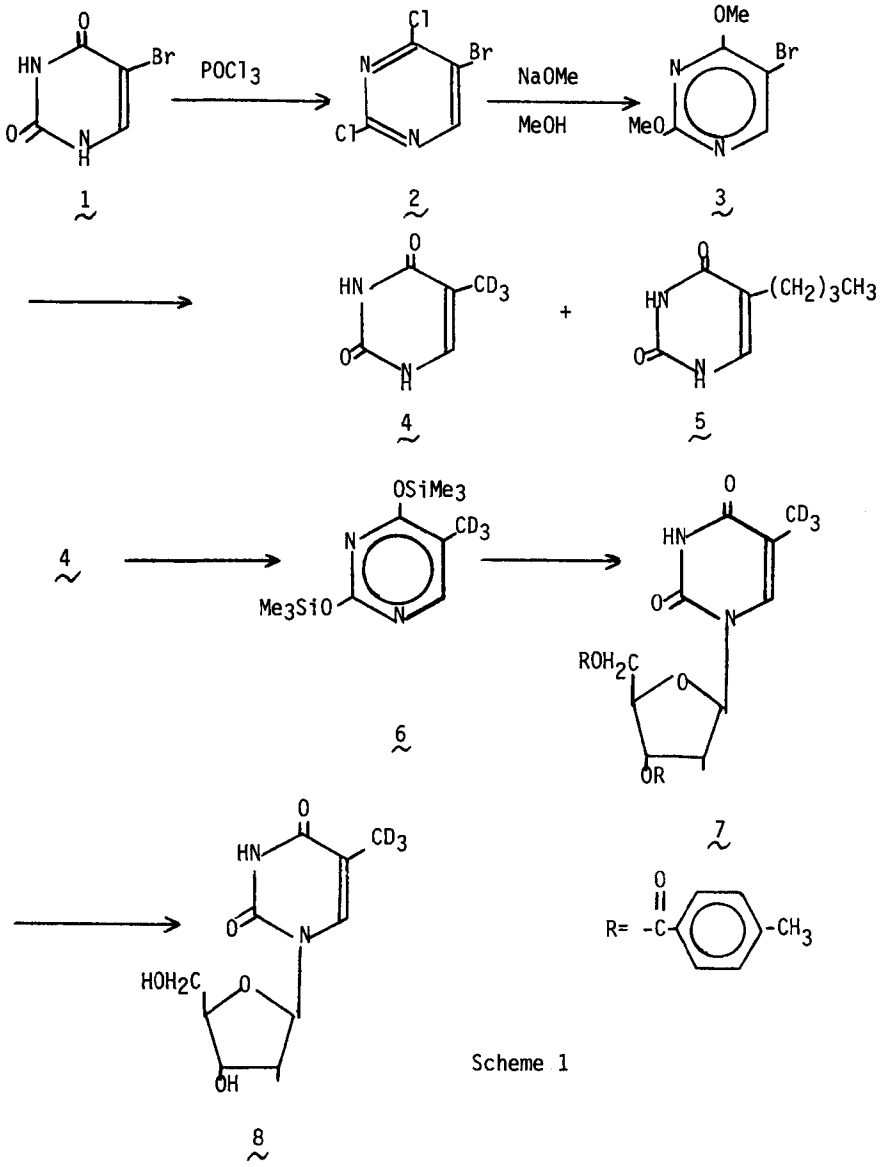
C-11 labeled thymidine and thymidylate have been synthesized enzymatically and used in metabolic studies (8,9). The C-14 analogs were synthesized from pyrimidine lithium salt with $^{14}CH_3I$ (10). However, the purification of 5-methyl-^{14}C-2'-deoxyuridine synthesized by this method was tedious and the yield was low. In fact, 5-methyl-^{14}C-2'-deoxyuridine synthesized by this method was not isolated but only detected and estimated by its UV spectrum. In order to have sizable quantities of deuterated thymine and thymidine for biological studies, these two compounds were synthesized by the method similar to that of Lawson (11) for the preparation of thymidine-1,3-^{15}N-6-^{13}C-α,α,α-d_3 with some modification.

Chlorination of 5-bromouracil (1) with $POCl_3$ gave 2,4-dichloro-5-bromopyrimidine (2) (12). Reaction of 2 with sodium methoxide gave 2,4-dimethoxy-5-bromouracil (3) (12). Treatment of 3 with n-BuLi followed by CD_3I gave, after hydrolysis, a mixture of 5-methyl-d_3-uracil (4) and 5-n-butylpyrimidine (5). Compound 4 was purified by fractional sublimation. The formation of compound 5 was apparently due to a coupling reaction. If solvent (THF) is not carefully dried, hydrolysis of the pyrimidine-lithium salt takes place and uracil becomes a major product.

Silyation of compound 4 with trimethylchlorosilane in hexamethyldisilazane (HMDS) gave compound 6 as an intermediate. Reaction of compound 6 with 1α-chloro-2-deoxy-3,5-bis(p-toluoyl)-α-D-ribofuranosyl chloride (13) in the presence of $SnCl_4$ (14) gave 5-methyl-d_3-2'-deoxyuridine ditoluoylate (7). The yield of compound 7 depends on the purity of compound 6. The presence of HMDS as an impurity retards the Friedel-Crafts reaction and hence reduces the yield of compound 7 dramatically. Hydrolysis of compound 7 with NaOMe-MeOH gave the final product: 5-methyl-d_3-2'-deoxyuridine (8) (see Scheme 1).

The structures of these compounds were verified by elemental analysis, UV, TLC, and NMR data and by comparison with authentic samples.

It is interesting to note that the chemical shifts of C-6 protons of compounds 3 - 4 change tremendously. This is due to the inductive effect of bromine as compared with methyl-d_3. In the case of compound 3, the chemical shift of C-6 proton is δ8.24, while the chemical shift of the corresponding proton of 2,4-dimethoxy-5-methyl-d_3-pyrimidine is δ7.88.



Scheme 1

In summary, the sequence described in Scheme 1 provides a convenient method for the synthesis of 5-methyl-d_3-uracil and 5-methyl-d_3-2'-deoxyuridine in sizable quantities.

EXPERIMENTAL

NMR spectra was measured on a JEOL MH-100 spectrometer and TMS used as an internal standard. Mass spectra were determined on a Hitachi Perkin-Elmer RMU-7 mass spectrometer.

The solvents were carefully purified: tetrahydrofuran was distilled from lithium aluminum hydride. 1,2-Dichloroethane was refluxed for 2 hours over P_2O_5 and distilled. Stannic chloride was distilled at atmospheric pressure. Methyl iodide-d_3 was purchased from Stohler Isotope Chemicals, Rutherford, NJ.

2,4-Dichloro-5-bromopyrimidine (2) (12)

Compound 2 was synthesized by the method of Jansen et al. from 5-bromouracil and phosphorus oxychloride in 58.4% yield. The compound was used in the next step without further purification.

2,4-Dimethoxy-5-bromopyrimidine (3) (12)

A solution of 3.8 g (165.2 mmol) of Na in 60 mL of MeOH was added into a solution of 10.4 g (45.84 mmol) of compound 2 in 20 mL of MeOH. The mixture was stirred at room temperature for 30 min, filtered, and the filtrate was evaporated to dryness. The residue was suspended in 50 mL of H_2O. The precipitates were filtered by suction, dried, and then sublimed at 100°C/3mm to give 8 g (80%) of compound 3, m.p. 64-65°C (lit. (12) m.p. 63-64°C); NMR ($CDCl_3$) δ8.24 (S,1H), 4.02 (S,3H), 3.96 (S,3H).

5-Methyl-d_3-Uracil (4)

A hexane solution of n-butyllithium (2.29 M, 7.5 mL) was added into a solution of 2,4-dimethoxy-5-bromopyrimidine (3) (3.46 g, 15.9 mmol) in 60 mL of tetrahydrofuran at -65°C under an atmosphere of nitrogen. Methyl iodide-d_3 (2.2 g, 15.18 mmol) in 5 mL of tetrahydrofuran was added to the orange-colored solution. The solution was stirred at -65°C for 1 1/2 hr and then excess of dry

ice was added. The mixture was stirred at room temperature for an additional hr and then poured into 50 mL of H_2O. The organic layer was separated and the aqueous layer was extracted with ether (3 x 50 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness to give 2,4-dimethoxy-5-methyl-d_3-pyrimidine. NMR ($CDCl_3$): δ7.88 (S,1H), 3.92 (S,3H), 3.88 (S,3H) and trace of butyl group.

The residue was dissolved in 50 mL of 6N HCl and refluxed for 2 hr. The solution was evaporated to dryness. The residue was suspended in ether, filtered, and then sublimed in vacuo to give 50 mg of 5-n-butyluracil (5) at 130°/3mm and 1.535 g (76.6%) of 5-methyl-d_3-uracil (4) at 230°/3mm. The mass spectrum gave a correct M^+ at m/e 129 for $C_5H_3D_3N_2O_2$. TLC (chloroform-methanol, 9:1, silica gel) showed a single spot at Rf 0.79. NMR (DMSO-d_6): δ10.90 (br,1H, exchangeable with D_2O), 10.50 (br,1H, exchangeable with D_2O), 7.20 (d,J=5Hz,1H).

2,4-Bis(Trimethylsilyloxy)-5-methyl-d_3-uracil (6)

The mixture of 367.60 mg (2.92 mmol) of compound 4, 10 mL of hexamethyldisilazane (HMDS) and 1 mL of trimethylchlorosilane was refluxed for 3 hr. Ammonia was vigorously evolved and NH_4Cl deposited in the condenser. The solution was evaporated and the oily residue was dried in vacuo to give 2,4-bis(trimethylsilyloxy)-5-methyl-d_3-uracil (6) as an intermediate. The compound was used in the next step without further purification. NMR ($CDCl_3$): δ7.84 (S,1H), 0.28 (S,18H).

5-Methyl-d_3-2'-deoxyuridine Ditoluoylate (7)

To the suspension of 343.98 mg (0.885 mmol) of 1α-chloro-2-deoxy-3,5-bis(p-toluoyl)-α-D-ribofuranosyl chloride and compound 6 in 20 mL of dry 1,2-dichloroethane cooled in an ice-bath, 0.2 mL of stannic chloride was added. The yellow solution was then stirred at room temperature overnight and then neutralized with saturated $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with methylene chloride. The combined

organic layer was dried (Na_2SO_4) and concentrated. The residue was recrystallized from ethanol to give 136.57 mg (9.73%) of compound 7, m.p. 198-200°C (lit. (11,15) 197°C, and 197-198°C). TLC (toluene-acetic acid-water, 5:5:1; silica gel) showed a single spot at Rf 0.79. NMR ($CDCl_3$): δ8.78 (br,1H), 7.80, 7.12 (A_2B_2,8H), 6.36 (t,J=6Hz,1H), 5.56 (br,1H), 2.40 (S,6H). The yield of compound 7 was not optimized.

5-Methyl-d_3-2'-deoxyuridine (8)

A solution of 69.86 mg (3.04 mmol) of Na in 10 mL of MeOH was added into the suspension of 321.7 mg (0.668 mmol) of 5-methyl-d_3-2'-deoxyuridine ditoluoylate (7) in 20 mL of MeOH. The solution was stirred at room temperature for 3 hr and then evaporated to dryness. The residue was washed with ether, dissolved in a small amount of MeOH and passed through a Dowex 50-X8 (H^+) column (2.5 x 12 cm). The compound was eluted with MeOH:H_2O (v/v 2:1) and the solvent was evaporated to dryness. The residue was washed with $CHCl_3$ several times and dried to give 125 mg (76%) of 8, m.p. 180-182°C (lit.(11,15) 187°C, and 183-184°C). TLC (toluene-acetic acid-water, 5:5:1, silica gel) showed a single spot at Rf 0.00 and at 0.81 in (methanol-chloroform, 1:4, silica gel). NMR (DMSO-d_6): δ7.66 (S,1H), 6.20 (t,J=6Hz), 2.12 (t,2H).

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