^{18}O LABELED NUCLEOSIDES. 1. A GENERAL METHOD FOR THE SYNTHESIS OF SPECIFICALLY LABELED PYRIMIDINE DEOXYRIBOSIDES

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SUMMARY

A facile method for the preparation of ^{18}O labeled pyrimidine deoxyribosides is described. The method provides a means of selectively labeling the base, sugar or both the base and sugar moieties with high levels of ^{18}O. Thymidine is used as a model to illustrate the method. The site and level of label was established using mass spectrometry.

Key Words: Nucleosides, Stable Isotopes, Mass Spectrometry

INTRODUCTION

Although a number of methods for the incorporation of deuterium into pyrimidine nucleosides have been reported (1-5), relatively few procedures (1,6-8) have described the labeling of nucleosides of any class with ^{18}O. Because of the growing use of stable isotopically labeled compounds in GC/MS studies (9,10), we have been attempting to develop general methods for the labeling of clinically important nucleosides (11). In this report we describe a facile method for the synthesis of specifically labeled ^{18}O analogs of pyrimidine deoxyribosides using thymidine as a model. The reaction can be extended to any deoxyriboside capable of forming an anhydro linkage. This synthetic scheme is based on the hydrolysis of anhydronucleosides in basic solution to afford products that are substituted in the aglycone with the nucleophile in the position originally involved in the anhydro linkage (12-14).

Thymidine was chosen as a model for the pyrimidine deoxynucleosides because it is readily available, inexpensive and is currently of considerable interest as an antitumor agent (15,16).

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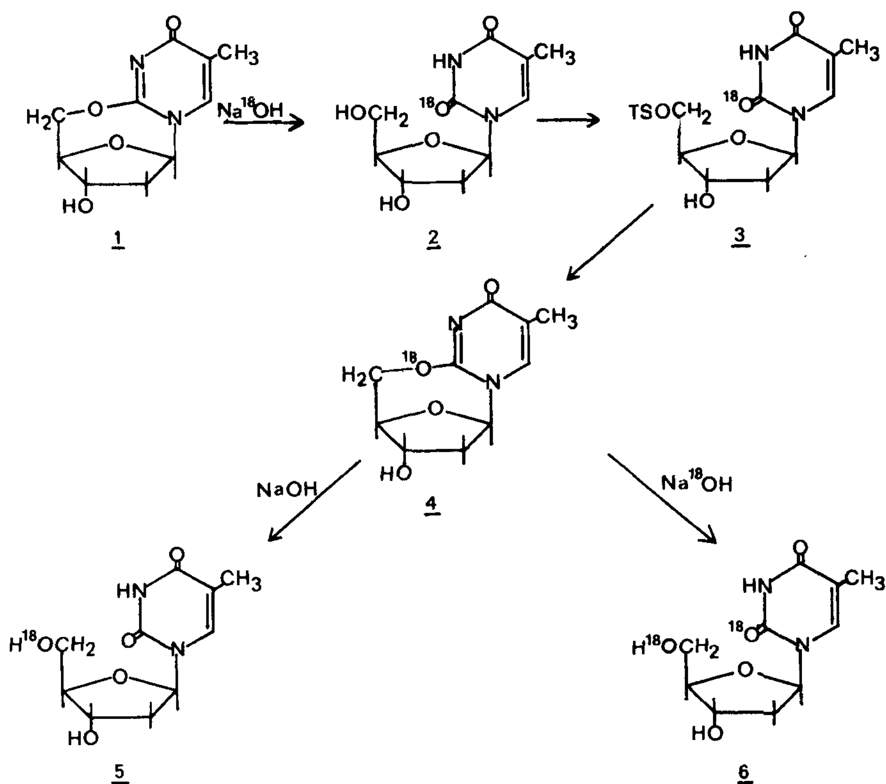
RESULTS AND DISCUSSION

Hydrolysis of the anhydro ring of O^2,5'-anhydrothymidine (1) (17) with $Na^{18}OH$ affords [O^2-^{18}O]thymidine (2) as shown below in the reaction scheme. Reformation of the anhydro bridge affords the key intermediate 4 which can again be ring opened to afford [5'-^{18}O]thymidine (5) when NaOH is used as the nucleophile. Alternatively, the di labeled [O^2,5'-$^{18}O_2$]thymidine (6) is the product when $Na^{18}OH$ is used to effect ring cleavage. The reaction sequence is therefore simple, is based on known nucleoside chemistry (17) and affords specifically labeled products with a high level of isotopic incorporation.

REACTION SCHEME



The site of label was determined by comparing the mass spectrum of 2, 5 and 6 with that of unlabeled thymidine. The molecular ion of thymidine is observed to shift from m/z 242 to m/z 244 in the mass spectra of 2 and 5 and to m/z 246 for 6.

These shifts indicate the incorporation of one ^{18}O atom into 2 and 5 and two ^{18}O atoms into 6. The B + H and B + 2H ions (18) of thymidine are observed to shift from m/z 126 and 127 to m/z 128 and 129 in 2 and 6 indicating that one ^{18}O is located in the heterocyclic portion of these molecules. Since this shift is not observed in 5, no label is present in the base moiety of this compound. However, the sugar ion at m/z 117 in thymidine has shifted to m/z 119 in both 5 and 6 which indicates the presence of ^{18}O in the carbohydrate. The O^2 and 5' positions may be confidently assigned as the substitution sites based on the well known chemistry of this reaction (12-14). The ions and the observed shifts used to assign the position of label are shown in Table 1. A more detailed discussion of the mass spectra of 2, 5 and 6 will be presented elsewhere.

Table 1. Ions Used to Assign Position of Label

Compound	Sugar ion (m/z)	B+H (m/z)	M+· (m/z)
Thymidine	117	126	242
2	117	128	244
5	119	126	244
6	119	128	246

The amount of label incorporated was determined by slow scanning of the molecular ion region of the samples in the chemical ionization mode (19). The use of CI was necessitated by the absence of strong ions in the molecular ion region with electron impact ionization of the free compounds or their TMS or permethyl derivatives (20). A comparison of the molecular ion region of thymidine with 2, 5 and 6 under these conditions is shown in Table 2. The results indicate that the amount of label incorporated is below that expected from the isotopic content of the $H_2{}^{18}O$ (99%) used to prepare the $Na^{18}OH$. The amount of unlabeled

Table 2. Molecular Ion Region of Labeled Compounds

m/z (Rel.Int.)	Thymidine (Ref)	2	5	6
240	-	-	-	-
241	0.84	-	-	-
242	8.26	-	-	-
243	100.00	8.00	2.10	-
244	13.31	8.49	3.37	-
245	6.05	100.00	100.00	7.25
246	-	14.60	11.07	4.78
247	-	2.84	3.09	100.00
248	-	-	-	11.80
249	-	-	-	3.26

thymidine in 2 is seen to be approximately 6% and this level carries through to compound 6. A potential major source of contamination is the presence of unreacted thymidine in either the tosyl derivative or the anhydro intermediate. This source of contamination is, however, eliminated from consideration based on product purity as evidenced by thin-layer chromatography and the melting point data. When a new sample of $Na^{18}OH$ was prepared for the synthesis of 5, the amount of label approached more closely the theoretical levels (98.74% ^{18}O incorporated) which indicates the inadvertent contamination of the $Na^{18}OH$ used to prepare 2 and 6. The amount of unlabeled thymidine in 6 is, however, undetectable so 6 is acceptable for quantitative studies.

Although a source of contamination has produced a lower level of incorporated isotope than expected, this method offers a synthetic route to labeled pyrimidine deoxynucleosides which are unavailable by any other means. These labeled compounds are unique in that the position of the isotopic label will permit the identification of the base and/or sugar portions of these compounds to be followed specifically, even in the event of cleavage of the glycosidic bond. Thus, pyrimidine deoxyribosides labeled as described in this report may prove of significant value in studying the complex biochemistry of this class of compounds in *in vivo* and *in vitro* systems.

EXPERIMENTAL

Mass spectra were obtained on a Varian 311A mass spectrometer operating under control of a Varian SS200 data system. Mass spectral conditions included a source temperature of 240°C, an ionizing voltage of 70 eV in the electron impact mode, a resolution of 1000 and a scan rate of 5 sec/decade. Chemical ionization conditions were as described by McCloskey and Wilson (19) with a scan rate of 25 sec/decade over the mass range 230 to 255 amu with methane being used as the reagent gas. The scans symmetrically distributed about the peak maximum were averaged to eliminate bias.

Thin-layer chromatography utilized silica gel GF plates (Analtech, Newark) 250 microns thick which were developed in chloroform methanol (4:1). Preparative thin layer chromatography was performed in a like manner except the stationary

phase was 1000 microns thick. All samples were homogenous after separation by preparative TLC.

All reagents were of commercial origin. Solvents were distilled and stored over 4Å molecular sieves.

Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected.

Oxygen-18 was purchased from Norsk-Hydro Sales, New York, and contained 99% $H_2{}^{18}O$.

$[O^2$-$^{18}O]$Thymidine (2). O^2,5'-Anhydrothymidine (1) (17) (200 mg, 0.89 mmol) was dissolved in 1.0 N $Na^{18}OH$ (400 µl) (prepared from 99% $H_2{}^{18}O$ by cautious addition of sodium metal) and the mixture stirred at room temperature for 24 h. The mixture was added to the top of a Dowex 50(H+) column (50 x 4 cm, 100-200 mesh) and the column washed with water (500 ml). The product was homogenous by TLC (R_f=0.57) and MS showed 92.8% incorporation of ^{18}O at C2 (7.2% unlabeled 1).

5'-O-Toluene Sulfonyl-$[O^2$-$^{18}O]$thymidine (3). p-Toluenesulfonyl chloride (140 mg, 0.73 mmol) was added to a stirred solution of 2 (160 mg, 0.66 mmol) in anhydrous pyridine (5 ml) at 0°C. The reaction was allowed to proceed for 12 h in the cold and an additional portion of tosyl chloride (25 mg, 0.13 mmol) was added. After an additional 36 h of stirring at 0°C, water (5 ml) was added and the mixture extracted with $CHCl_3$ (3x10 ml). The extracts were combined and washed successively with saturated $NaHCO_3$ (15 ml) and water (15 ml). The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness *in vacuo*. The residue was dissolved in a small amount of $CHCl_3$ and applied to a preparative TLC plate. After development the band corresponding to 3 (R_f=0.67) was scraped and eluted with MeOH to afford a 27% yield of 3 (70 mg, 0.18 mmol). This material was used without further purification. Mp 170-172°C (dec.) [lit. (17) mp 171-176°C (dec.)].

O^2,5'-Anhydro-$[O^2$-$^{18}O]$thymidine (4). A stirred solution of 3 (65 mg, 0.16 mmol) and 1,5-diazabicyclo[5,4,0]undec-5-ene (DBU) (Aldrich) (300 mg, 1.97 mmol) in anhydrous acetonitrile (5 ml) was heated at reflux temperature for 24 h. The reaction was filtered hot and the filtrate taken to dryness *in vacuo*. The residue was dissolved in a small amount of $CHCl_3$ and separated by preparative TLC

(R_f=0.22) to give 4 (20 mg, 0.09 mmol) in 54% yield.

[5'-^{18}O]Thymidine (5). The anhydrothymidine 4 (8 mg, 0.03 mmol) was hydrolyzed with freshly prepared 1N NaOH (20 µl) following the procedure described for 2 above. The product 5 was isolated from TLC (R_f=0.57) in 66% yield (4 mg, 0.02 mmol). MS showed 98.7% ^{18}O was incorporated in the sugar (1.3% unlabeled thymidine).

[O^2,5'-^{18}O]Thymidine (6). Following the same procedure as for 5, 4 (8 mg, 0.03 mmol) was hydrolyzed with 1N Na^{18}OH (20 µl) to give 6 (4 mg, 0.02 mmol) (R_f=0.57) in 65% yield. MS showed an isotope distribution of 93.2% ^{18}O and 6.8% ^{18}O. The amount of unlabeled thymidine was <0.5%.

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