EFFICIENT SYNTHESIS OF SPECIFICALLY DEUTERATED NUCLEOSIDES: PREPARATION OF 4'-DEUTERIOTHYMIDINE[1]

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Abstract - A very straightforward synthesis of 4'-deuteriothymidine (1) from inexpensive thymidine (2) is described. The key step involves triple deprotonation of the ester prepared by selective oxidation of unprotected thymidine (and esterification) and deuteration from the α-face to produce the desired 4'-deuterio derivative (5) which is then taken on to 1. Thus one can prepare the 4'-deuteriothymidine (1) in only five steps from thymidine (2) in an overall yield of 16%.

Chemical nucleases, synthetic or naturally occurring, usually cleave DNA by oxidizing the sugar unit.[3] The first step generally involves the abstraction of a hydrogen atom from the 2'-deoxyribose unit, although the site of the attack varies. Specifically isotopically-labelled nucleotides are valuable tools for studying the mechanism of cleavage by a chemical nuclease. In particular, 4'-labelled nucleotides have been used in the study of the chemical nucleases that first abstract the hydrogen atom from the 4'-position. Neocarzinostatin exhibited kinetic isotope effects of 2 to 5 when reacted with 4'-labelled nucleotides[4] while the deuterium atom on C4' was transferred to reduced calicheamycin.[5] For a project aimed at determining the mechanism of the cleavage of nucleic acids by the system ortho-phenanthroline, copper, hydrogen peroxide,[6] we needed to prepare a small DNA fragment that incorporated specifically deuterated nucleosides. For this reason, we required a sample of thymidine which was specifically deuterated at the 4'-position, 1. This compound is known in the literature but the reported preparation which uses an enzyme based method seemed inappropriate for our purpose.[7] Also the best chemical method among several developed[8] for the production of specifically deuterium-labelled nucleosides, the work of Townsend and coworkers,[8b] did not appear to be particularly applicable for the synthesis of 1. Therefore we developed a new route to 1 which begins with thymidine (2) itself. We described herein this approach, which should also be applicable to the preparation of other normal and 2'-deoxynucleosides.
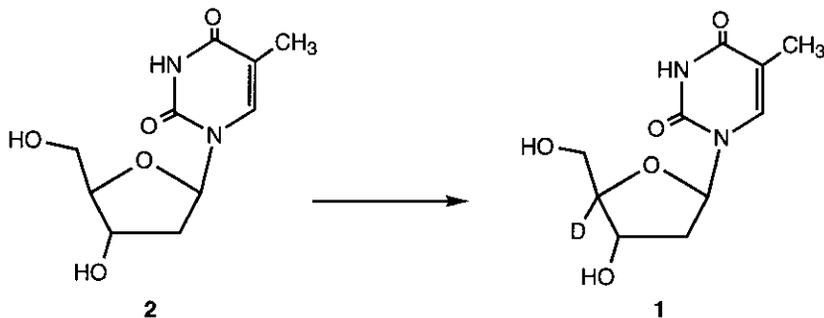
Of the several possible syntheses of 4'-deuteriothymidine (**1**), we chose to investigate the simple deprotonation-deuteration of the 5'-carboxylic ester, which could be easily prepared by selective oxidation of the nucleoside at the 5'-position, namely the primary alcohol in the presence of the secondary one. If the introduction of deuterium could be carried out with high chemo- and stereoselectivity, then this route would be quite short and would probably represent one of the most efficient ways of preparing 4'-deuterated nucleosides. It was our expectation that, after the first two deprotonations on the acidic imide and hydroxyl groups, the third deprotonation would occur at the proton α to the carboxylic ester to give the trianion. This anion α to the ester should not eliminate the 3'-alkoxy group since the dianions of other simpler β-hydroxy esters had proven stable to β-elimination.[9,10] Steric hindrance from the pyrimidine base oriented on the β-face at the anomeric 1'-carbon would be expected to favor reprotonation (or deuteration) from the α-face although the 3'-alkoxide on the α-face might well hinder that process. After deuteration, hydride reduction of the ester would regenerate the primary alcohol (after a final exchange of the deuterium atoms on the heteroatoms for hydrogen). This approach of selective oxidation, deprotonation, deuteration, and final ester reduction was accomplished in a straightforward manner as shown in Scheme 1.

Selective oxidation of thymidine (**2**) into the 5'-carboxylic acid (**3**) in 62% yield and its conversion to the methyl ester (**4**) in 85% yield was already known.[11] The key step involved the triple deprotonation of this ester (**4**) and stereoselective deuteration of the resulting trianion. We found that a large excess of base and a very polar aprotic solvent were necessary in order to guarantee deprotonation α to the ester. After considerable experimentation, it was found that a mixture of LDA and *n*-butyllithium in THF containing some HMPA proved to be best. Thus treatment of a solution of **4** in 88:12 THF:HMPA cooled to -78 °C with 5 equiv of LDA followed by warming to 25 °C, recooling to -78 °C and addition of 4 equiv of *n*-butyllithium afforded a red solution.[12] After allowing the solution to stir for 1 h at -78 °C to ensure deprotonation, excess D_2O/AcOD were added. It proved impossible to isolate the desired product (**5**) cleanly after this step due to the presence of the HMPA and solubility problems. Therefore the entire mixture was treated

with acetic anhydride and pyridine to produce the acetate (6). Again although crude spectroscopic data indicated the presence of 6, there were problems in isolating the compound from the HMPA in a pure state so it was taken on directly. Hydride reduction[13] of 6 followed by heating in acidic methanol to remove the boric acid and borate salts, afforded, after an extended purification process (see Experimental Section), the desired 4'-deuteriothymidine (1) in an overall yield of 30% from the starting ester (4). No attempts have been made to optimize this yield. Thus 4'-deuteriothymidine (1) is available from thymidine (2) in five steps in 16% overall yield. The efficiency of the deuterium incorporation was determined to be very high in the crude products (5) and (6) and in the final product (1) by both proton and carbon NMR. Thus the proton and carbon NMR spectra indicated the near-total disappearance of the signals due to the 4'-hydrogen and the 4'-carbon atom (due to reduced intensity caused by coupling with D, reduced nuclear Overhauser effects, and presumably long relaxation times), implying that the extent of deuteration was >95%.
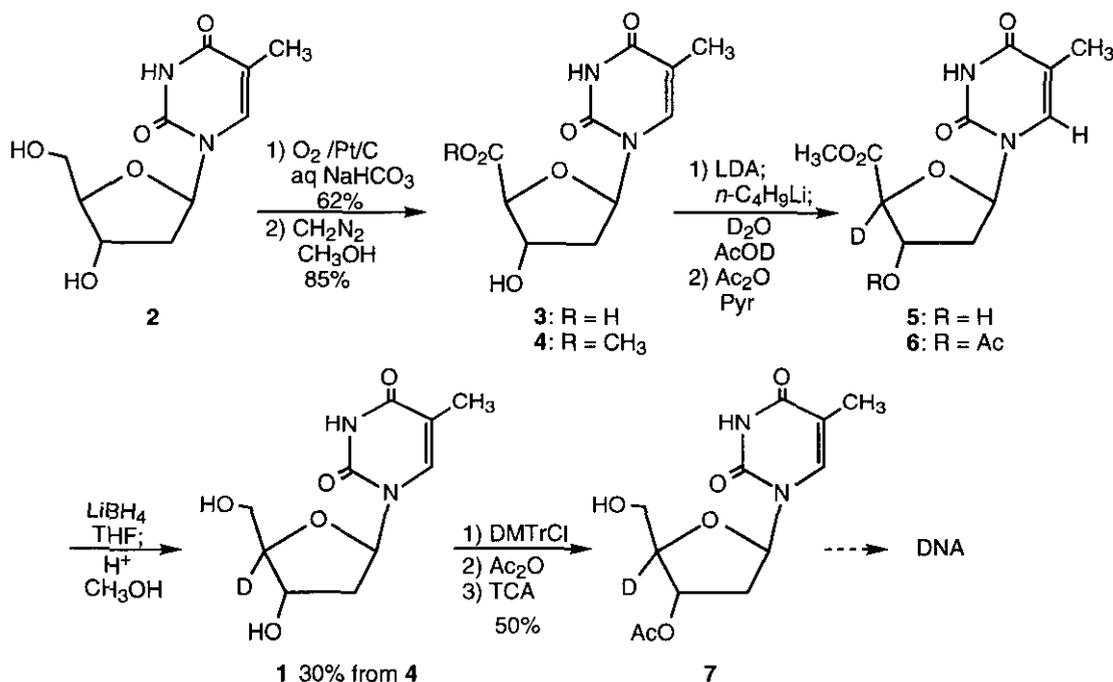
Scheme 1

We believe that the stereoselectivity seen in this alkylation can be explained by the following hypothesis. The enolate would preferentially exist in the C3'-endo conformation which would place the 3'-hydrogen in a pseudoaxial arrangement (see structure 8). Attack of the enolate on the electrophile would occur in the staggered axial orientation due to torsional strain, e.g., on the bottom face as shown.[14] In this conformation, the steric hindrance due to the pyrimidine base at the anomeric carbon is also probably larger than that of the alkoxide at C3'. So for these two reasons, only the α-substituted product is observed.

In order to prepare the required 5'-triphosphate[15] of (**1**) for use in the enzymatic synthesis of the required

DNA segment, we converted (**1**) into the 3'-acetate by the usual three-step process, namely 5'-dimethoxy-

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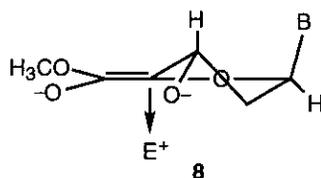
tritylation, 3'-acetylation, and final acidic detritylation. This sequence furnished the acetate (**7**) in 50%

unoptimized yield. This compound was then used to prepare the triphosphate and from it the DNA. The

results of the biochemical experiments, namely the elucidation of the mechanism of the nuclease activity,

will be described in due course.[16]

Finally it is important to point out that a minor by-product in our early attempts at effecting 4'-deuteration

was the 4',6-dideuteriothymidine (**9**) which indicates that the 6-proton is also acidic enough to be removed

under these conditions. We are currently examining the synthesis of other labelled nucleosides for use in

determining the mechanism of chemical nucleases. We are also investigating the use of this process for the

production of other 4'-substituted nucleosides, e.g., 4'α-azido, hydroxyalkyl and acylated systems.[10]

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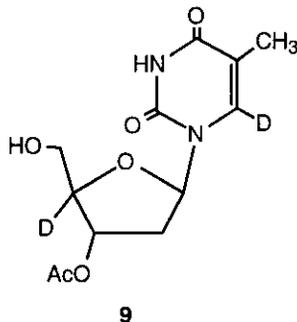
EXPERIMENTAL

Melting points were recorded on a Büchi micro capillary melting point apparatus and are uncorrected. [1]H

NMR were recorded on a Bruker AC-200 spectrometer, operating at 200.132 MHz, and ^{13}C NMR spectra

were also recorded on the AC-200 operating at 50.323 MHz. Chemical shifts are reported as δ values

(ppm) relative to either internal TMS or the solvent peak, coupling constants are reported in Hertz (Hz), and

the normal abbreviations are used. IR spectra were recorded on a Nicolet 510 FTIR with the data reported

in wave numbers (cm^{-1}). Flash column chromatography was performed on Fluka or EM Science silica gel

60 (40-63 μm). Solvent systems are reported as either volume:volume mixtures or volume percent

mixtures. Thin layer chromatography was performed using Merck silica gel 60 F_{254} 0.2 mm plates.

Concentration *in vacuo* or at reduced pressure refers to removal of solvent by use of a Büchi rotary evaporator with a heated water bath under water aspirator pressure. All reagents and solvents were purchased commercially and used without further purification except for the following: HMPA was stored over 3Å molecular sieves and tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl.

Thymidine-5'-carboxylic Acid (3). Platinum metal (6 g, 5% on activated carbon) was suspended in a solution of 1.5 g of $NaHCO_3$ in 300 mL of water in a round bottomed flask. The suspension was stirred under a hydrogen atmosphere at 70 °C for 15 min. The hydrogen was then removed and 3 g of solid thymidine (**2**) was added. After the system was purged with pure oxygen, the flask was equipped with an oxygen balloon, and the suspension was stirred vigorously at 70 °C overnight. The reaction was cooled to 25 °C, the solution was filtered, and the platinum on carbon was recovered on a piece of fine filter paper. The solution was neutralized by passing it through an acidic ion exchange column (Amberlite IR-120 in H^+ form). After concentration *in vacuo* and refrigeration overnight, the product crystallized and was collected by filtration to yield 1.86 g of **3**, 62% yield. mp 263-264 °C (lit. mp 263-265 °C).[11] ^{1}H NMR (DMSO-d_6): 11.36 (s, 1H, H3), 8.09 (br d, 1H, J = 0.6 Hz, H6), 6.35 (dd, 1H, J = 9.0, 5.4 Hz, H1'), 5.81 (br s, 1H, OH), 4.46 (br d, 1H, J = 4.7 Hz, H3'), 4.33 (s, 1H, H4'), 2.15 (dd, 1H, J = 13.2, 5.5 Hz, H2'$_a$), 1.97 (ddd, 1H, J = 13.3, 9.0, 4.7 Hz, H2'$_b$), 1.79 (s, 3H, CH3). ^{13}C NMR (DMSO-d_6): 172.80, 163.98, 150.73, 136.53, 109.61, 85.68, 84.68, 73.97, 12.64 (one high field carbon not resolved due to overlap with DMSO). IR (KBr): 3422, 3387, 1692, 1651 cm^{-1}.

Methyl thymidine-5'-carboxylate (4). To a vigorously stirred solution of 1.4 g (5.47 mmol) of **3** in 150 mL of methanol was added slowly an ethereal solution of diazomethane prepared from 8.03 g (54.7 mmol) of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG, Aldrich) at 50 °C. The product started crystallizing as the ethereal solution of diazomethane was added. After concentrating the solution, additional product crystallized and was filtered to give 1.2 g of the ester **4**, 85% yield. mp 255-257 °C (lit., mp 247 °C).[16] ^{1}H NMR (DMSO-d_6): 11.38 (br s, 1H, H3), 7.96 (br s, 1H, H6), 6.37 (dd, 1H, J = 8.8, 5.7 Hz, H1'), 5.87 (d, 1H, J = 3.7 Hz, OH), 4.49 (br s, 1H, H3'), 4.42 (s, 1H, H4'), 3.74 (s, 3H, COOCH3), 1.90-2.25 (m, 2H, H2'), 1.81 (s, 3H, CH3). ^{13}C NMR (DMSO-d_6): 171.65, 163.80, 150.69, 136.23, 109.73, 85.69, 84.50, 73.81, 52.43, 38.14, 12.57. IR (KBr): 3410, 1713, 1662 cm^{-1}.

Methyl 4'-deuteriothymidine-5'-carboxylate (5). The ester (**4**) (300 mg, 1.11 mmol) was dissolved in 35 mL of dry 12% HMPA in THF. The solution was stirred at -78 °C under argon, and lithium diisopropylamide (3.7 mL, 1.5 M, 5.55 mmol) was added slowly. The mixture was allowed to warm to 25

°C and cooled back to -78 °C within 1 h. *n*-Butyllithium (2.6 mL, 1.7 M, 4.42 mmol) was added slowly

with the solution turning red during the addition. After the mixture was stirred for 1 h at -78 °C, 0.5 mL of

D_2O and 1 mL of AcOD were added to quench the reaction and to make the pH of the solution between 5

and 6. The insolubility of **5** in most organic solvents and the high boiling point of HMPA made it difficult

to purify the product. After removing the volatile solvents *in vacuo*, the remaining mixture was coevapor-

ated with dry pyridine and was used directly in the next reaction. When pure THF was used as the solvent

in earlier attempts at this deprotonation-deuteration, a large amount of THF was required to dissolve **4**.

However, **5** was isolated, albeit in low yield. ^{1}H NMR (DMSO-d_6): 11.38 (br s, 1H, H3), 7.96 (br s, 1H,

H6), 6.37 (dd, 1H, J = 8.8, 5.7 Hz, H1'), 5.87 (d, 1H, J = 3.7 Hz, OH), 4.49 (br s, 1H, H3'), 4.42 (s,

< 0.05H, H4'), 3.74 (s, 3H, COOCH$_3$), 1.9-2.25 (m, 2H, H2'), 1.81 (s, 3H, CH$_3$). ^{13}C NMR (DMSO-

d_6): 171.65, 163.80, 150.69, 136.23, 109.73, 85.69, 73.81, 52.43, 38.14, 12.57. (The carbon signal

due to C4' ppm was not observed.) IR (KBr): 3411, 1713, 1680, 1663 cm^{-1}.

Methyl 3'-*O*-acetyl-4'-deuteriothymidine-5'-carboxylate (6). To the mixture obtained from last

reaction containing **5** was added excess pyridine and acetic anhydride. The reaction was kept in a refrigera-

tor overnight and then quenched with water and methanol. Evaporation of the solvents gave a residue to

which ethanol and toluene were added and the solution reevaporated. This was repeated several times. The

residue was taken up in 200 mL of chloroform. The organic layer was washed successively with water, 0.1

M sulfuric acid, water, saturated sodium bicarbonate, and brine. Final evaporation of the solvents *in vacuo*

gave a mixture whose ^{1}H NMR showed the presence of the product (**6**), along with HMPA and some

minor impurities. The HMPA was hard to remove either by aqueous extraction or silica gel column

chromato-graphy. Therefore the mixture was used directly in the next reaction. However, an NMR of crude

6 could be obtained. ^{1}H NMR (CDCl$_3$): 8.55 (br s, 1H, NH), 8.05 (d, 1H, J = 1.2 Hz, H6), 6.52 (dd,

1H, J = 9.4, 5.2 Hz, H1'), 5.44 (d, 1H, J = 5.1 Hz, H3'), 4.60 (s, 1H, H4'), 3.85 (s, 3H, COOCH$_3$),

2.50 (dd, 1H, J = 9.8, 5.2 Hz, H2'$_a$), 2.02 - 2.25 (m, 1H, H2'$_b$), 2.15 (s, 3H, CH$_3$COO), 1.98 (s, 3H,

CH$_3$). ^{13}C NMR (CDCl$_3$): 170.79, 169.86, 163.81, 150.68, 135.67, 111.68, 86.07, 81.81, 75.90,

52.88, 36.32, 20.83, 12.61.

4'-Deuteriothymidine (1). After coevaporating the mixture from the previous reaction containing **6**

with benzene several times to remove any moisture, 2 mL of dry THF and 2.5 mL of 2 M LiBH$_4$ in THF

were added. The reaction was heated at 65 °C for 2 h and then methanol and water were added. Solvents

were evaporated *in vacuo*, and the residue was taken up in methanol and passed through an acidic ion

exchange column (Amberlite IR-120 in its H$^+$ form). The solution was repeatedly coevaporated with methanol to remove the boric acid. The residue then contained mainly the desired product and HMPA. Flash column chromatography on silica gel separated these with HMPA being eluted first with 10% MeOH in chloroform. The product was then eluted with 20% MeOH in chloroform to yield 90 mg of 1, 30% from 4. ^{1}H NMR (CD$_3$OD): 7.81 (d, 1H, J = 1.0 Hz, H6), 6.28 (t, 1H, J = 6.8 Hz, H1'), 4.40 (dd, J = 5.3, 4.2 Hz, H3'), 3.91 (m, < 0.05H, H4'), 3.81 (d, 1H, J = 12.0 Hz, H5'$_a$), 3.72 (d, 1H, J = 12.0 Hz, H5'$_b$), 2.20 - 2.27 (m, 2H, H2'), 1.88 (s, 3H, CH$_3$). ^{13}C NMR (CD$_3$OD): 166.36, 152.32, 138.13, 111.49, 86.23, 72.11, 62.73, 41.12, 12.43 (the carbon signal due to C4' was not observed). IR (KBr): 3316, 1707, 1700, 1667 cm^{-1}.

3'-O-Acetyl-4'-deuteriothymidine (7). A solution of 6 (100 mg, 0.41 mmol) and dimethoxytrityl chloride (280 mg , 0.82 mmol) were stirred in 2 mL of dry pyridine for 8 h at 25 °C. Excess acetic anhydride was then added, and the reaction was stirred overnight at 25 °C. Ethanol and water was added. Solvents were evaporated *in vacuo*, and pyridine was carefully removed by repeated coevaporation with ethanol and toluene. The residue was dissolved in a few drops of dichloromethane, and 1 mL of 5% trichloroacetic acid in dichloromethane was added. The solution instantly turned red and was quickly loaded onto a silica gel column. Elution with a gradient of 5% to 10% methanol in chloroform afforded 60 mg of 7, 50% yield. ^{1}H NMR (CD$_3$OD): 7.84 (d, 1H, J = 0.9 Hz, H6), 6.27 (t, 1H, J = 7.2 Hz, H1), 5.30 (dd, 1H, J = 4.9, 3.1 Hz, H3'), 4.08 (m, < 0.05H, H4'), 3.80 (s, 2H, H5'), 2.29 - 2.39 (m, 2H, H2'), 2.09 (s, 3H, CH$_3$COO), 1.88 (s, 3H, CH$_3$). ^{13}C NMR (CD$_3$OD): 172.20, 166.22, 152.56, 137.88, 111.84, 86.17, 76.34, 62.88, 38.38, 20.86, 12.42 (the carbon signal due to C4' was not observed). IR (KBr): 3416, 1686 cm^{-1}.

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