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A REGIO AND STEREOSELECTIVE SYNTHESIS OF 2',2'',3',4'-TETRADEUTERIO-2'-DEOXY NUCLEOSIDES

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Abstract: Methyl β-D-arabinopyranoside (1) has been stereoselectively deuterated, by the treatment of deuterated Raney Ni in D_2O, to give the 2,3,4-trideutereo-β-D-arabinopyranoside 2 in 33% yield. It was then converted to 3,4-isopropylidene derivative 3b in 90% yield and subsequently derivatized to 2-O-phenylthiocarbonate 4 (85%). Compound 4 was deoxygenated at C-2 and was isolated as 2:8 (α:β) anomeric mixture 6 in 70% yield (based on 4 in two steps). Compound 6 was converted to the free sugar 7, by the treatment of 0.8 M aqueous HCl, which was then successively converted to pentofuranosides: 8 → 9 → 10; the crystalline α-chlorosugar 10 was thus obtained in 60% yield in four steps starting from 6. Subsequently α-chlorosugar 10 was coupled to different nucleobases of DNA to give 2',2'',3',4'-tetradeuterio-2'-deoxynucleosides in moderate yields.

Nuclear magnetic resonance (NMR) spectroscopy has proved to be a very powerful tool for understanding the conformation and dynamics of single and double stranded DNA[1-3]. Despite all currently available techniques to estimate the phosphate backbone conformation, population of the pseudorotamers[4] or the handedness[5] of a DNA molecule by assigning chemical shifts, coupling network of each constituent sugar and estimating interproton distances between each nucleotide residues by NOE measurements, it is still difficult to assess the mobilities of each sugar unit of an oligonucleotide larger than a hexadecamer. An examination of the ^{1}H-NMR spectra of an oligomer reveals that it is clearly because of the severe spectral overlap, mainly, of the H-2' and H-2'' and H-5' and H-5'' resonances from sugar moieties of the oligo-DNA molecule. The problem due to the overlapping of these sugar protons could partly be solved by the selective suppression of absorption(s) at a suitably chosen site in an oligo-DNA by substituting proton(s) with deuterium(s) while observing the full J coupled net work in the unsubstituted part of the oligo-DNA. Such a selective suppression of information may be useful, particularly, in a long oligo-DNA molecule. Danyluk and coworkers[6-10] have selectively deuterated one or more residues in a given segment of nucleic acid which has led to the unequivocal assignment of signals to each base and sugar ring proton, leading to a convenient determination of the spin-spin coupling constants. This group of workers has used labeled mononucleotides, extracted from fully deuterated blue-green algae, *synechococcus lividus*, grown in D_2O. But, the biochemical ways of deuterium incorporation into a nucleoside are limited by the fact that the molecule becomes fully deuterated and when incorporated into an oligonucleotide, the informations due to this particular unit is completely suppressed.

An alternative approach to the solution of the problem of overlapping could be the incorporation of deuterium regioselectively at a particular sugar carbon of a monomer unit. As for example, the

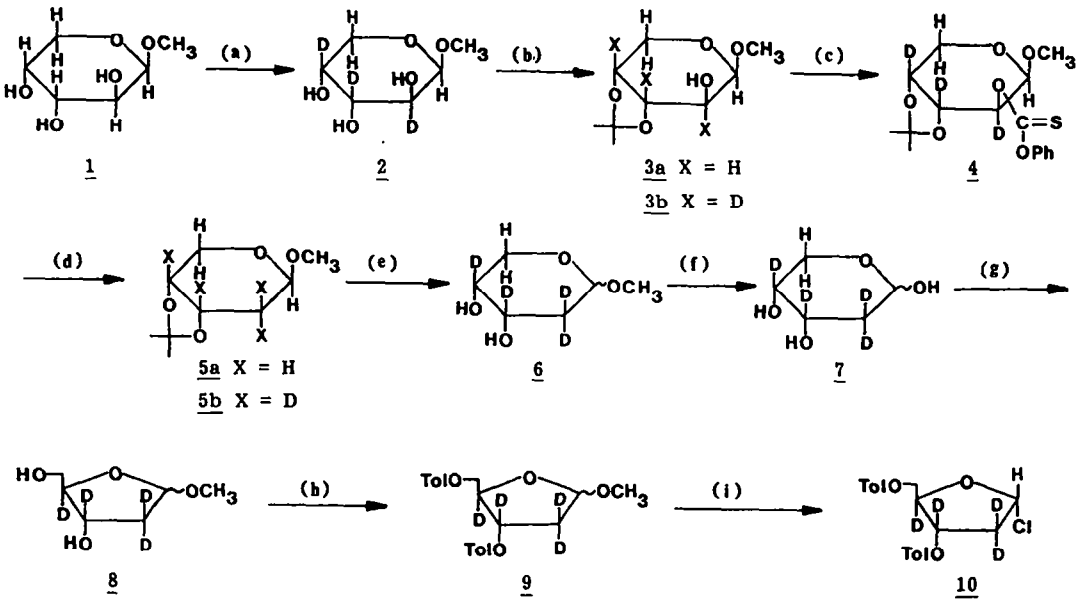
complexity occurring from the absorptions of H-2' and H-2'' (5 spin-spin couplings) of a deoxynucleoside could be reduced by substituting either H-2' or H-2'', or both, with deuterium(s) while the information from the other regions of the same sugar moiety could still be obtained. This chemoselective approach would be also useful for understanding the fine details of conformational behaviour of oligonucleotides in ^{13}C-NMR spectroscopy[11-14] by detecting a particular carbon absorption more easily amongst overlapping or nearly overlapping resonances.

Synthesis of the ^{2}H-2' or ^{2}H-2''[15], ^{2}H-2'/^{2}H-2''[16] and ^{2}H-5'/^{2}H-5''[17] have been already reported. Presently available method[18] to incorporate ^{2}H-4' affords a mixture of C-4' diastereoisomers from which the desired compound is obtained only in a poor yield.

We herein report a synthesis of 2',2'',3',4'-tetradeuterio-2'-deoxynucleosides which are envisioned to be useful, when they are selectively incorporated into an oligo-DNA molecule, due to the following reasons: first, any weak NOE between the nucleobase and the sharp singlet from its H-1' would be more easily detectable to determine the handedness[5] of the oligo-DNA chain and secondly, although, the coupling between H-4' and H-5'/5'' is completely lost in such a molecule, the H-5'/5'' gem coupling and their coupling with phosphorous are still available for studies on the phosphate backbone conformations[1-10].

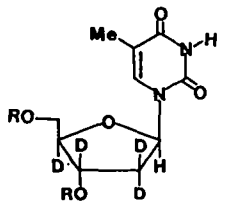
We reasoned that a prerequisite step for the synthesis of such stereo and regiospecifically labelled 2'-deoxynucleosides should be to incorporate deuteriums specifically at C-2, C-3 and C-4 of a pentofuranose and subsequently condense the corresponding chlorosugar 10 with the appropriate nucleobase. Koch and his coworkers[19-21] have introduced deuterated Raney nickel and D_2O as a versatile reagent for exchanging protons with deuterium at different positions of a pyranose. The yields of the deuterated compounds prepared using latter procedures are reported to be moderate. The rates of exchange of protons at different carbons of the pyranose or furanose are also found to be very different and is also completely dependent on the structure of a particular pyranose or furanose sugar. It, however, turned out that it is the best and cheapest method available so far for introducing an unlimited number of deuteriums in a particular molecule depending on the number of hydroxyl functionalities available in it.

Isomerically pure methyl β-D-arabinopyranoside (1)[22], D_2O and deuterated Raney nickel were heated under reflux for 15-20 h to give a mixture of compounds, from which the desired compound 2 could be isolated in 33% yield. No attempt was made to identify all compounds formed in the latter reaction. It was, however, clear that extensive anomerisation took place at C-4 of compound 1 to give 20 (ca. 40-50%), the structure of the latter compound was based on the fact that it did not undergo isopropylidenation reaction. Reduction of reaction time to 10 h did not exchange the C-2 proton completely while the amount of the anomarised product 20 remained quite comparable. This is because of the fact[23] that the H-4 exchanged at a much faster rate than the H-2. Isopropylidenation[24] of compound 2 was performed by stirring a mixture of 2, 2,2-dimethoxypropane and a catalytic amount of p-toluenesulfonic acid monohydrate at 20 °C for 3 h to give compound 3b in 90% yield. Conversion of 3b to the 2-deoxysugar 5b was attempted through intermediates 4 and 21b. Compound 21b could be prepared[25] in good yield from 3b by treating it successively with NaH, CS_2 and CH_3I. But the attempted reduction of non-deuterated compound 21a to 5a with tri-n-butyltinhydride in toluene for 16 h at reflux temperature gave a mixture of compounds from which 5a could not be isolated. Compound 3b was, therefore, converted to the corresponding phenoxythiocarbonate 4 in 85% yield by the treatment of phenoxythiocarbonyl chloride in acetonitrile in presence of N,N-dimethylaminopyridine for 16 h at 20 °C. When a solution of compound 4 in toluene was treated with tri-n-butyltindeuteride at 70-80 °C for 2 h in presence of 2,2'-azo-bis(2-methylpropionitrile)[26] gave an almost quantitative conversion to the corresponding 2'-deoxysugar 5b. However, attempts to isolate compound 5b from the latter reaction mixture failed as it was always contaminated with some unidentified tin compounds. The crude reaction product was, therefore, treated with 80% aqueous acetic acid for 15 h at room temperature to give an anomeric mixture (α:β ca. 2:8) of the compound 6 which were isolated in 70% yield (based on 4) upon an aqueous extraction of the ether solution. Compound 6 was subsequently converted to the free sugar 7, upon treatment with 0.8 M HCl aqueous hydrochloric acid for 40 h at room temperature, which was transformed into the chlorosugar 10 in 60% yield following a standard procedure[27].

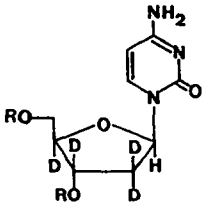


(a) Deuterated Raney nickel/D_2O; (b) 2,2-Dimethoxypropane/Ts-OH/DMF; (c) Phenoxythiocarbonyl-Cl/4-DMAP/Acetonitrile; (d) Bu_3SnX/AIBN/Toluene; (e) 80% Acetic acid; (f) 0.5M HCl; (g) MeOH/HCl; (h) Toluoyl-Cl/Pyridine; (i) HCl/Acetic acid

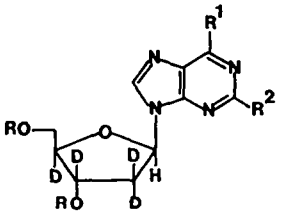
All reactions described above were performed with the pure compound 2 and are documented in the experimental section as "Procedure A". For practical purposes, it was not necessary to crystallize out compound 2, because it could be isolated easily as its isopropylidene derivative 3b (accompanied with two other minor products), from the unreacted sugars. All other subsequent reactions could, therefore, be performed with the crude mixture of 3b and they are described as "Procedure B". In case of "Procedure B", no attempt was made to isolate the pure products because at the end of the synthesis the chlorosugar 10 crystallized out in a pure state (37% overall yield in four steps from 6).



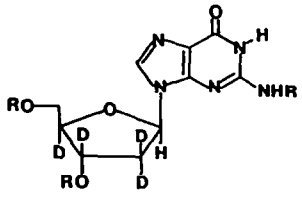
11 R = Toluoyl
12 R = H



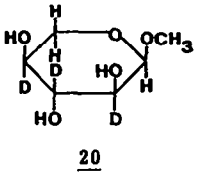
13 R = Toluoyl
14 R = H



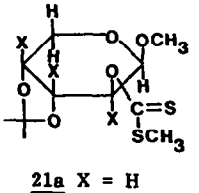
15 R = Toluoyl, R^1 = Cl, R^2 = H
16 R = H, R^1 = NH_2, R^2 = H
17 R = Toluoyl, R^1 = Cl, R^2 = NH_2



18 R = H
19 R = Acetyl



20



21a X = H
21b X = D

The chlorosugar 10 was then condensed[15,16] with thymine, 6-chloropurine and 2-amino-6-chloropurine to give the corresponding 2'-deoxy-2',2'',3',4'-tetradeuterio-nucleosides 11, 15 and 17. The condensation of 10 and cytosine[15,16] gave an inseparable mixture of 13 and the α-isomer. Therefore, the mixture was deprotected directly and the free nucleoside 14 was separated on a Dowex $^-$OH column. Compounds 11, 15 and 17 were deprotected and/or converted to the free nucleosides 12, 16 and 18. Compound 18 was isolated as its triacetate 19.

The ^{1}H-NMR spectra of the 2'-deoxy-2',2'',3',4'-tetradeuterio-nucleosides are shown in panel B while the corresponding non-deuterated (natural) nucleosides are shown in panel A in figures 1, 2, 3 and 4. A comparison of these spectra clearly illustrates the specific labellings achieved in the present work leaving, as expected, H-1' as a singlet and H-5',5'' as a double doublet.

EXPERIMENTAL

Melting points were uncorrected. ^{1}H-NMR spectra at 90 MHz and ^{13}C NMR at 23.7 MHz were recorded with Jeol FX 90Q instrument. Tetramethylsilane and CH_3OD were used as internal standards for $CDCl_3$ and D_2O solutions, respectively, except in case of compound 6 where acetonitrile was used as internal standard. The chemical shifts were reported in ppm (δ scale). UV absorption spectra were recorded with a Varian-Cary 2200 instrument; Jeol DX 303 instrument was used for recording mass spectra. Thin-Layer chromatography was performed on Merck, pre-coated 60 F_{254} plate and Merck Kieselgel G was used for short column chromatography. Specific rotations were recorded with a Perkin-Elmer 241 polarimeter.

Preparation of the deuterated Raney nickel: Raney nickel (20 ml, settled volume) was washed with deuterium oxide (8 x 4 ml of ca. 80% D_2O and then 8 x 4 ml of 99.8% D_2O). During the washings with 99.8% D_2O, Raney nickel was allowed to stand in it for half an hour between each washing. 20 ml of the deuterated Raney nickel was sufficient for the exchange reaction with 20 mmol of sugar 1 if Raney nickel used for deuteration is freshly prepared.

Methyl β-D-arabinopyranoside-2,3,4-$^2H_3$ (2). To a solution of methyl β-D-arabinopyranoside (1) (1.65 g, 10 mmol) in deuterium oxide (10 ml), deuterated Raney nickel (10 ml, settled volume) was added and the mixture was heated under reflux for 15 h. The mixture was cooled and Raney nickel was filtered off through a celite bed. The residue was washed several times with water. The filtrate and the washings were pooled together and it was evaporated to dryness. Products from two other batches were pooled together and the mixture was crystallized from ethanol. Yield: 1.6 (33%), m.p. 165-6 °C. ^{1}H-NMR (D_2O): 4.8 (s, 1H) H-1; 3.7 (q, $J_{5,5'}$ = 12.9 Hz, 2H) H-5,5'; 3.4 (s, 3H) OCH_3. ^{13}C-NMR (D_2O): 100.3 (d, J_{CH} = 170 Hz) C-1; 62.9 (t, J_{CH} = 147 Hz) C-5; 55.6 (q, J_{CH} = 143 Hz) OCH_3. MS (FAB^-): $(M-H)^-$ calc. 166.0795, found 166.0857. Calc. for $C_6H_9D_3O_5$: C, 43.11; H/D, 9.04. Found: C, 43.25; H/D, 9.17. $[\alpha]_D^{24}$ -229.8° (c 1.0, water)[28].

3,4-O-Isopropylidene methyl-β-D-arabinopyranoside-2,3,4-$^2H_3$ (3b). Procedure A. A mixture of compound 2 (1.7 g, 10 mmol), 2,2-dimethoxypropane (3.7 ml, 30 mmol) and p-toluenesulfonic acid monohydrate (20 mg) in dry N,N-dimethylformamide (20 ml) was stirred at 20 °C. After 3 h, ammonia was added to neutralise the acid. All volatile matters were removed in vacuo (bath temperature; 50 °C) and co-evaporated a few times with water. The syrupy residue was purified by column chromatography to give an oil. Yield: 1.8 g (90%). ^{1}H-NMR ($CDCl_3$): 4.7 (s, 1H) H-1; 3.9 (s, 2H) H-5,5'; 3.4 (s, 3H) OCH_3; 2.53 (s, 1H, exchangable) 2-OH; 1.53 and 1.36 (2s, 3H each) isopropylidene protons. ^{13}C-NMR ($CDCl_3$): 109.1 (s) methine carbon of isopropylidene; 99.0 (d, J_{CH} = 164.8 Hz) C-1; 59.3 (t, J_{CH} = 145.0 Hz) C-5; 55.6 (q, J_{CH} = 143.9 Hz) methoxy; 28.0 (q, J_{CH} = 125.6 Hz), 26.1 (q, J_{CH} = 127.0 Hz) methyl. MS (FAB^-): $(M-H)^-$ calc. 206.1108, found 206.1095.

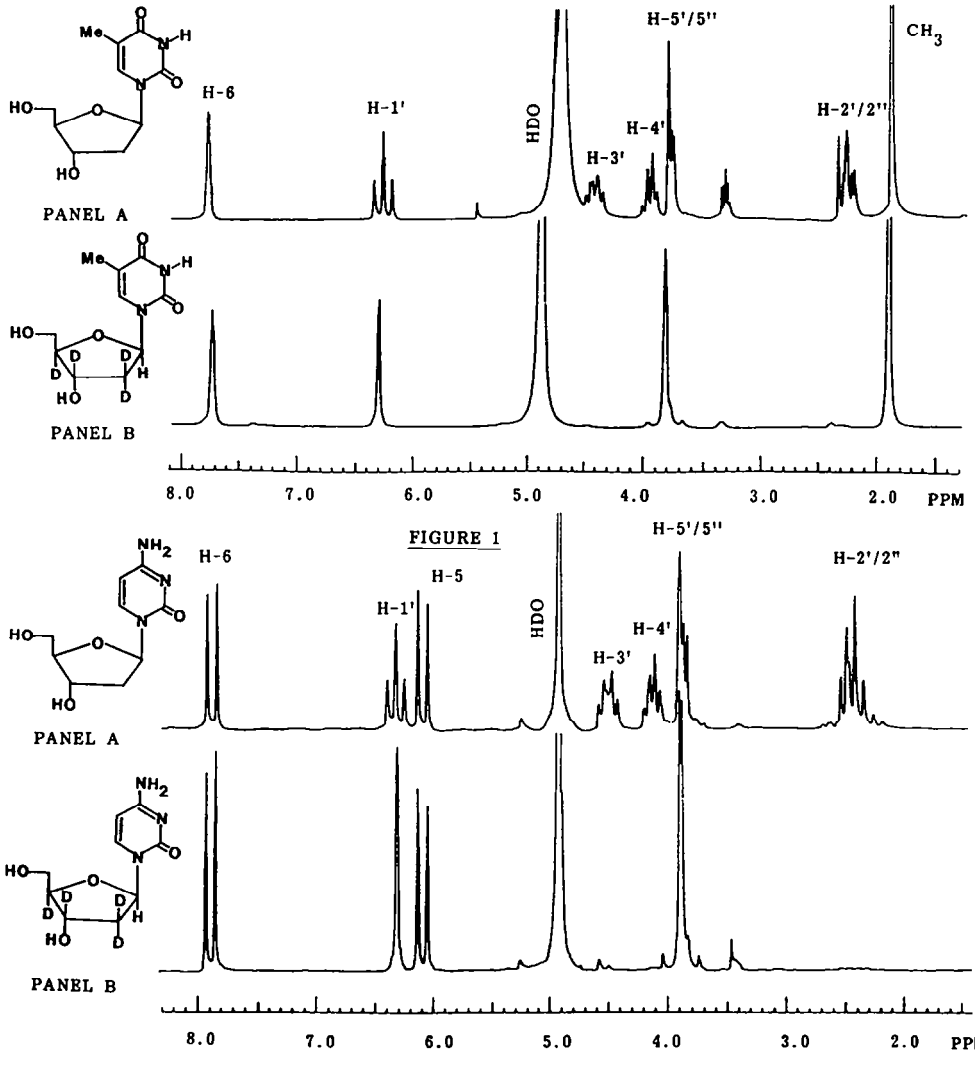
Procedure B: Methyl β-D-arabinopyranoside (1) (5.74 g, 35 mmol) was treated with deuterated Raney nickel and deuterium oxide in four batches as described above. The combined residue obtained was dried by coevaporation with acetonitrile and converted to the isopropylidene derivative. Volatile matters from the latter reaction mixture was evaporated and the residue was dissolved in water and

extracted with chloroform (6 x 25 ml). The organic phases were pooled together and evaporated to dryness to give an oil. Yield: 3 g (42%, calculated on the basis of 1).

2-O-Phenoxythiocarbonyl-3,4-O-isopropylidene methyl-β-D-arabinopyranoside-2,3,4-$^2H_3$ (4). Procedure A: A mixture of compound 3b (1.5 g, 7.2 mmol), N,N-dimethylaminopyridine (2.2 g, 17.6 mmol) and phenoxythiocarbonyl chloride (2.1 ml, 11.5 mmol) in dry acetonitrile (70 ml) was stirred overnight at 20 °C. The mixture was evaporated to dryness and purified by column chromatography to give a yellow oil. Yield: 2.1 g (85%). ^{1}H-NMR ($CDCl_3$): 7.3 (m, 5H) aromatic protons; 5.06 (s, 1H) H-; 4.02 (dd, $J_{5,5'}$ = 13 Hz, 2H) H-5,5'; 3.45 (s, 3H) OCH_3; 1.6 and 1.4 (2s, 3H each) isopropylidene group. ^{13}C-NMR ($CDCl_3$): 153.3, 129.4, 126.5, 121.8 (aromatic carbons); 96.1 (d, J_{CH} = 171.8 Hz) C-1; 58.3 (t, J_{CH} = 147.1 Hz) C-5; 55.7 (q, J_{CH} = 142.6 Hz) methoxy. MS (FAB$^-$): $(M-H)^-$ calc. 342.1091, found 342.1133.

Procedure B: Yield: 3.2 g (86%).

Methyl 2-deoxy-ribopyranoside-2,2',3,4-$^2H_4$ (6). Procedure A: Compound 4 (5 g, 14.6 mmol) was dissolved in dry toluene (290 ml) and nitrogen was bubbled through it for 20 min. Tri-n-butyltin-deuteride (7.3 ml, 23.3 mmol) and 2,2'-azo-bis-(2-methylpropionitrile) (470 mg) were added. The mixture was heated at 80 °C for 2 h, cooled and all volatile matters were removed in vacuo. The



oily residue was loaded on a silica gel column in hexane and the column was eluted in the same solvent to remove most of the tin containing compound. The column was then washed with ether to eluate the reaction product. All appropriate fractions were pooled together, evaporated and the oily residue was taken up in 80% acetic acid. After stirring the mixture at 20 °C for 15 h, volatile matters were removed in vacuo and coevaporated with toluene. The residue was taken up in ether and extracted with water (3 x 20 ml). Aqueous extracts were pooled together and evaporated to dryness. The residue was triturated with ether to give a white solid which was a mixture of α and β anomers in ca. 2:8 ratio. Yield: 1.61 g (72%); ^{1}H-NMR (D_2O): β anomer: 4.82 (s, 1H) H-1; 3.68 (q, $J_{5,5'}$ = 12.5 Hz, 2H) H-5,5'; 3.29 (s, 3H) OCH_3. α-anomer: 4.75 (s, 1H) H-1; 3.33 (s, 3H) OCH_3. ^{13}C-NMR (D_2O) β-anomer: 99.1 (d, J_{CH} = 170 Hz) C-1; 63.1 (t, J_{CH} = 146.5 Hz) C-5; 55.1 (q, J_{CH} = 144.2 Hz) OCH_3; α-anomer: 100.3 (d, J_{CH} = 170 Hz) C-1; 62.8 C-5; 55.6 OCH_3. MS (FAB$^-$): $(M-H)^-$ calc. 151.0909, found 151.0924. Calc. for $C_6H_8D_4O_4$: C, 47.35; H/D, 10.59. Found: C, 47.48; H/D, 10.74.

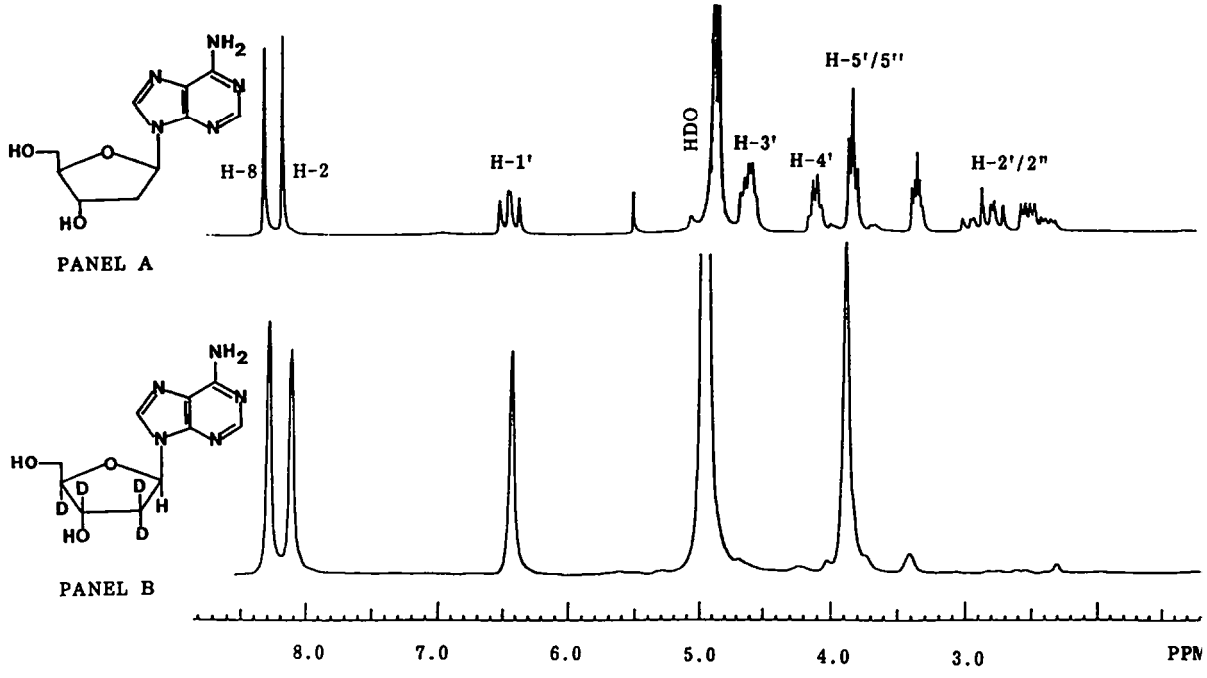


FIGURE 3

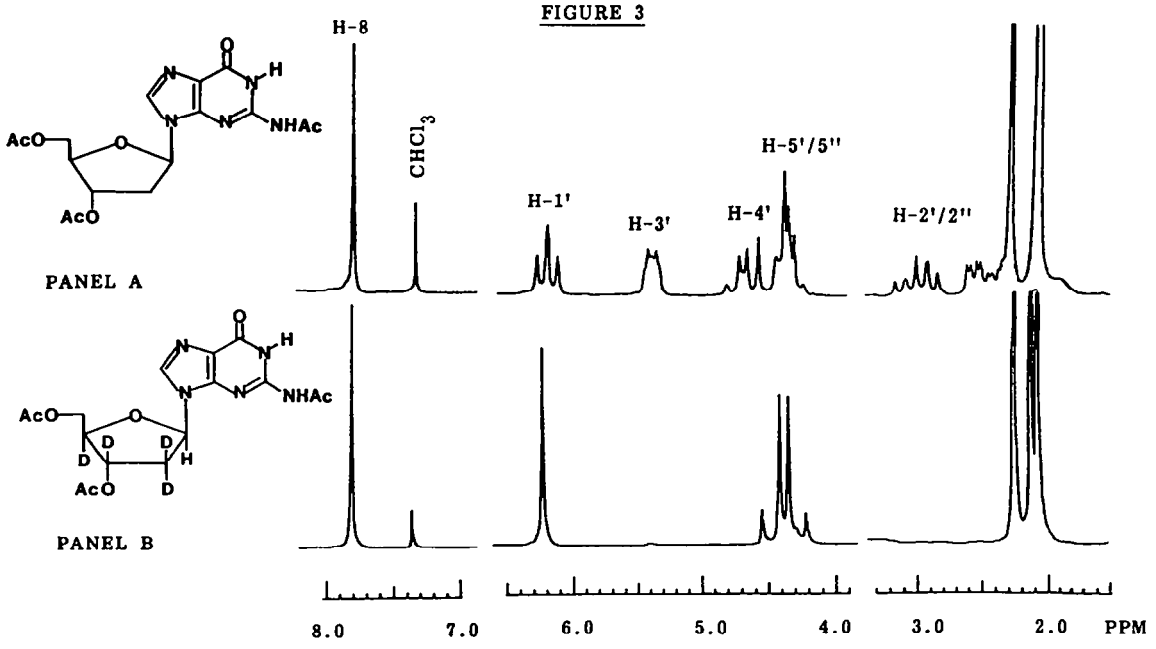


FIGURE 4

Procedure B: Yield: 0.96 g (67%).

Methyl 2-deoxy-3,5-di-O-p-toluoyl-D-erythro-pentose-2,2',3,4-$^2H_4$ (9). Procedure A: Compound 6 (1.61 g, 10.6 mmol) was treated with aqueous hydrochloric acid solution (0.8 M, 50 ml) for 40 h at 20 °C. The solution was neutralised with amberlite ($^-$OH), filtered and evaporated to dryness. The free sugar was converted to the title compound following a literature procedure[27] and isolated as a non-crystallizeable oil. Yield: 3.6 g (87%). ^{1}H-NMR ($CDCl_3$): α-anomer: 7.9-7.25 (m, 8H) aromatic; 5.18 (s, 1H) H-1; 4.57 (m, 2H) H-5,5'; 3.42 (s, 3H) methoxy; 2.4 (s, 6H) arom. methyl; β-anomer: 7.9-7.25 (m, 8H) aromatic; 5.22 (s, 1H) H-1; 4.5 (m, 2H) H-5,5'; 3.35 (s, 3H) methoxy; 2.4 (s, 6H) arom. methyl. ^{13}C-NMR ($CDCl_3$): α-anomer: 104.9 (d, J_{CH} = 170.9 Hz) C-1; 64.2 (t, J_{CH} = 149.0 Hz) C-5; 54.9 (q, J_{CH} = 141.6 Hz) methoxy. β-anomer: 105.5 (d, J_{CH} = 170.9 Hz) C-1; 65.1 (t, J_{CH} = 149.0 Hz) C-5; 54.9 (q, J_{CH} = 141.6 Hz) methoxy. MS (FAB$^-$): $(M-H)^-$ calc. 387.1746, found 387.1725. Calc. for $C_{22}H_{20}D_4O_6$: C, 68.02; H/D, 7.26. Found: C, 68.24; H/D, 7.16.
Procedure B: Yield: 3.1 g (92%).

2-Deoxy-3,5-di-O-p-toluoyl-D-erythro-pentosyl chloride-2,2',3,4-$^2H_4$ (10). Procedure A: Yield: 2.78 g (60%); m.p.: 117-8 °C; ^{1}H-NMR ($CDCl_3$): 7.95-7.28 (m, 8H) aromatic protons; 6.46 (s, 1H) H-1; 4.63 (q, $J_{5,5'}$ = 12.2 Hz, 2H) H-5,5'; 2.42 (s, 6H) methyl protons. ^{13}C-NMR ($CDCl_3$): 95.3 (d, J_{CH} = 186.5 Hz) C-1; 63.5 (t, J_{CH} = 149.4 Hz) C-5. MS (FAB$^+$): $(M-Cl)^+$ calc. 357.1640, found 357.1665. Calc. for $C_{21}H_{17}D_4ClO_5$: C, 64.2; H/D, 6.4; Cl, 9.02. Found: C, 64.11; H/D, 6.4; Cl, 9.23.
Procedure B: Yield: 1.17 g (37%).

1-(2'-Deoxy-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione-2',2'',3',4'-$^2H_4$ (11). M.p.: 195-6 °C. ^{1}H-NMR ($CDCl_3$): 8.98 (bs, 1H) NH; 7.94 (m, 4H) aromatic; 7.26 (m, 5H) aromatic and H-6; 6.46 (s, 1H) H-1'; 4.71 (q, $J_{5',5''}$ = 12.2 Hz, 2H) H-5',5''; 2.43 (s, 6H) methyl; 1.62 (s, 3H) 5-CH_3. ^{13}C-NMR ($CDCl_3$): 134.4 (d, J_{CH} = 177.3 Hz) C-6; 111.6 (s) C-5; 84.9 (d, J_{CH} = 168.4 Hz) C-1'; 64.2 (t, J_{CH} = 148.9 Hz) C-5'; 12.2 (q, J_{CH} = 128.9 Hz) 5-CH_3. MS (FAB$^-$): $(M-H)^-$ calc. 481.1913, found 481.1900. Calc. for $C_{26}H_{22}D_4N_2O_7$: C, 64.72; H/D, 6.26; N, 5.8. Found: C, 64.62; H/D, 6.43; N, 5.93.

Thymidine-2',2'',3',4'-$^2H_4$ (12). M.p.: 183 °C (MeOH). UV (EtOH): λ_{max} = 266 nm (ε = 8,200) pH 7. ^{1}H-NMR (D_2O + CD_3OD): 7.76 (s, 1H) H-6; 6.34 (s, 1H) H-1'; 3.88 (t, $J_{5',5''}$ = 13.3 Hz, 2H) H-5',5''; 1.97 (s, 3H) methyl. ^{13}C-NMR (D_2O + CD_3OD): 138.6 (d, J_{CH} = 180.6 Hz) C-6; 112.4 (s) C-5; 86.3 (d, J_{CH} = 169.7 Hz) C-1'; 62.4 (t, J_{CH} = 142.8 Hz) C-5'; 12.8 (q, J_{CH} = 128.8 Hz) methyl. MS (FAB$^-$): $(M-H)^-$ calc. 245.1076, found 245.1136. Calc. for $C_{10}H_{10}D_4N_2O_5$: C, 48.77; H/D, 7.36; N, 11.37. Found: C, 48.91; H/D, 7.42; N, 11.46. $[\alpha]_D^{24}$ +16.7° (c 1.0, water)[28].

2'-Deoxycytidine-2',2'',3',4'-$^2H_4$ (14). M.p.: 200-2 °C (MeOH). UV (H_2O): λ_{max} = 271 nm (ε = 7,700) pH 7. ^{1}H-NMR (D_2O + CD_3OD): 7.9 (d, $J_{5,6}$ = 7.4 Hz) H-6; 6.31 (s, 1H) C-1'; 6 $J_{5,6}$ = 7.4 Hz) H-5; 3.86 (q, $J_{5',5''}$ = 12.6 Hz, 2H) H-5',5''. ^{13}C-NMR (D_2O + CD_3OD): 142.7 (d, J_{CH} = 181.9 Hz) C-6; 97.1 (d, J_{CH} = 175.2 Hz) C-5; 87.4 (d, J_{CH} = 170.1 Hz) C-1'; 62.5 (t, J_{CH} = 142.8 Hz) C-5'. MS (FAB$^-$): $(M-H)^-$ calc. 230.1079, found 230.1110. Calc. for $C_9H_9D_4N_3O_4$: C, 46.75; H/D, 7.40; N, 18.17. Found: C, 46.63; H/D, 7.61; N, 18.35. $[\alpha]_D^{24}$ +56.3° (c 0.8, water)[28].

6-chloro-9-(2'-deoxy-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)purine-2',2'',3',4'-$^2H_4$ (15). M.p.: 107-8 °C (methanol). ^{1}H-NMR ($CDCl_3$): 8.67 (s, 1H) H-8; 8.29 (s, 1H) H-2; 7.92-7.21 (m, 8H) arom.; 6.57 (s, 1H) H-1'; 4.72 (q, $J_{5',5''}$ = 12.2 Hz, 2H) H-5',5''; 2.44 (s, 3H); 2.40 (s, 3H) toluoyl-methyl. ^{13}C-NMR ($CDCl_3$): 85.4 (d, J_{CH} = 166.0 Hz) C-1'; 63.6 (t, J_{CH} = 149.5 Hz) C-5'. MS (FAB$^+$): $(M+H)^+$ calc. 511.1686, found 511.1675. Calc. for $C_{26}H_{19}D_4ClN_4O_5$: C, 61.11; H/D, 5.32; Cl, 6.94; N, 10.96. Found: C, 61.22; H/D, 5.4; Cl, 6.98; N, 11.12.

2'-Deoxyadenosine-2',2'',3',4'-$^2H_4$ (16). M.p.: 185-6 °C (water). UV (EtOH): λ_{max} = 259 nm (ε = 14,100) pH 7. ^{1}H-NMR (D_2O + CD_3OD): 8.27 (s, 1H) H-8; 8.1 (s, 1H) H-2; 6.42 (s, 1H) H-1'; 3.88

(t, $J_{5',5''}$ = 13.3 Hz, 2H) H-5'. ^{13}C-NMR (D_2O + CD_3OD): 153.6 (d, J_{CH} = 202.6 Hz) C-8; 141.5 (d, J_{CH} = 214.8 Hz) C-2; 86.3 (d, J_{CH} = 167.2 Hz) C-1'; 63.2 (t, J_{CH} = 142.8 Hz) C-5'. MS (FAB^+): $(M+H)^+$ calc. 256.1348, found 256.1311. Calc. for $C_{10}H_9D_4N_5O_3$: C, 47.05; H/D, 6.71; N, 27.43. Found: C, 47.12; H/D, 6.8; N, 27.31. $[\alpha]_D^{24}$ -26.4° (c 0.5, water)[28].

2-Amino-6-chloro-9-(2'-deoxy-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)purine-2',2'',3',4'-$^2H_4$ (17). M.p.: 176-7 °C (ethanol). ^{1}H-NMR ($CDCl_3$): 7.89 (m, 5H) aromatic and H-8; 7.25 (m, 4H) aromatic; 6.37 (s, 1H) H-1'; 4.73 (q, $J_{5',5''}$ = 12.0 Hz, 2H) H-5',5''; 2.43 and 2.39 (two s, 6H) toluoyl-methyl. ^{13}C-NMR ($CDCl_3$): 84.9 (d, J_{CH} = 164.8 Hz) C-1'; 63.8 (t, J_{CH} = 150.1 Hz) C-5. MS (FAB^+): $(M+H)^+$ calc. 526.1796, found 526.1766. Calc. for $C_{26}H_{20}D_4ClN_5O_5$: C, 59.37; H/D, 5.36; Cl, 6.74; N, 13.31. Found: C, 59.5; H/D, 5.48; Cl, 6.91; N, 13.18.

2-N,3',5'-O-triacetyl-2'-deoxyguanosine-2',2'',3',4'-$^2H_4$ (19). M.p.: 195 °C (ethanol); UV (EtOH) λ_{max} = 256 nm (ε = 13,600) pH 7. ^{1}H-NMR ($CDCl_3$ + CD_3OD): 7.82 (s, 1H) H-8; 6.23 (s, 1H) (q, $J_{5',5''}$ = 11.8 Hz, 2H) H-5',5''; 2.28 (s, 3H) N-2-acetyl; 2.14 and 2.09 (two s, 6H) 3' and 5'-O-acetyl. ^{13}C-NMR ($CDCl_3$ + CD_3OD): 138.2 (d, J_{CH} = 213.6 Hz) C-8; 85.2 (d, J_{CH} = 166.0 Hz) C-1'; 63.7 (t, J_{CH} = 148.9 Hz) C-5'. MS (FAB^+): $(M+H)^+$ calc. 398.1614, found 398.1653. Calc. for $C_{16}H_{15}D_4N_5O_7$: C, 48.36; H/D, 5.83; N, 17.62. Found: C, 48.47; H/D, 6.03; N, 17.73. $[\alpha]_D^{24}$ -14.0° (c 0.4, 99.9% ethanol + chloroform)[28].

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28. Specific rotations of the corresponding non-deuterated compounds under the same conditions are as follows: compound 1 (-231.8°), thymidine (+17.4°), 2'-deoxycytidine (+56.8°), 2'-deoxyadenosine (-27.2°) and 2-N,3',5'-O-triacetyl-2'-deoxyguanosine (-14.1°). Thus, a comparison of the specific rotations of compound 2 and its non-deuterated analogue, compound 1, unequivocally assigns the stereochemistry of C-2, C-3 & C-4 of 2. Furthermore, a similar comparison of specific rotations of final deuterated compounds 12, 14, 16 & 19 with those of the non-deuterated (natural) nucleosides confirm our configurational assignments.