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REGIOSPECIFIC SYNTHESIS OF 2'-DEOXY-2',2''-DIDEUTERIO NUCLEOSIDES

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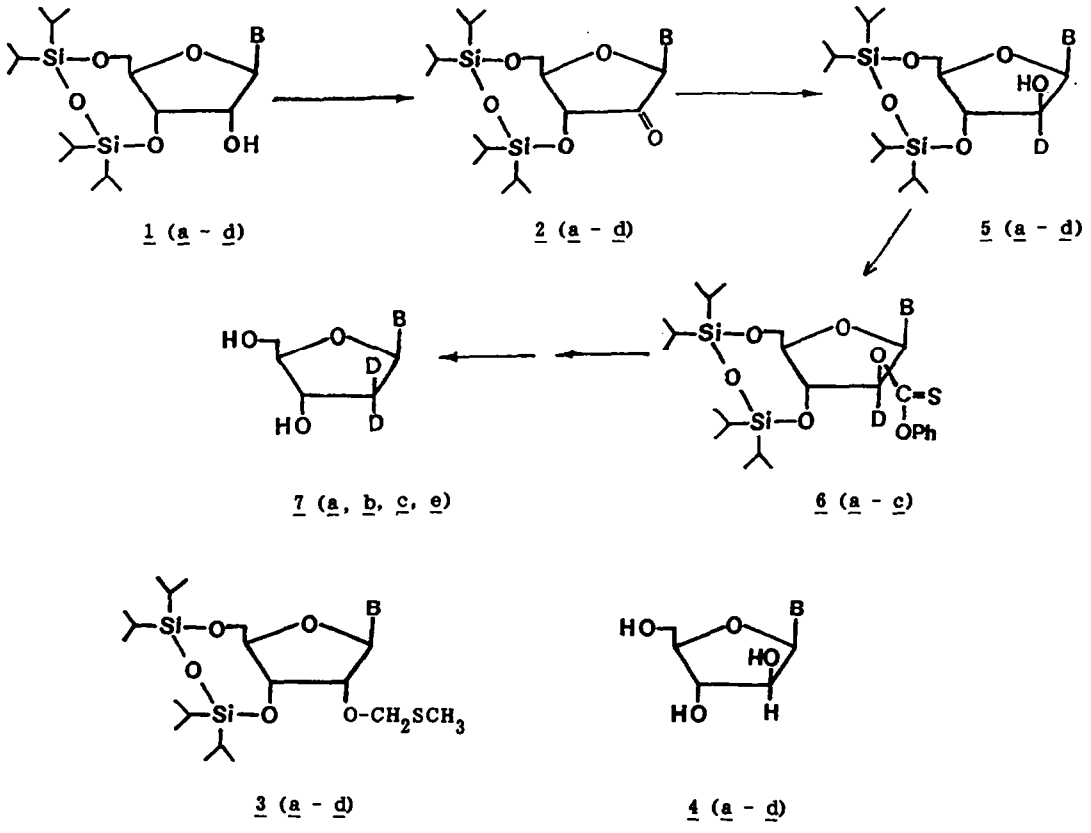
Summary: Two routes have been devised for the first synthesis of 2',2''-dideuterio-2'-deoxynucleosides. First route involves the oxidation of an appropriately 3',5'-protected ribonucleosides with CrO_3-pyridine-acetic anhydride complex, followed by direct $NaBD_4$ or $LiAlD_4$ reduction of the ketone to give 2'-deuterio arabinonucleosides (5a-5c) (ca. 60% in two steps). Compounds 5a-5c were then derivatized to 2'-O-thiocarbonates 6a-6c in ca. 70% yields which were then subjected to Bu_3SnD reduction and F^- ion treatment to give 2'-deoxy-2',2''-dideuterio adenosine, uridine and cytidine in good yields. In the second route, benzyl 3,4-isopropylidine-β-D-arabinopyranoside 9 was converted to the 2-ketosugar 10 (88%) which was reduced to give crystalline 2(R)-deuterio-alcohol 11 (94%). Compound 11 was then converted to benzyl 3,4-isopropylidene-2-deoxy-2-dideuterio-β-D-ribo-pyranoside 13 (79%) by Bu_3SnD promoted cleavage of the thiocarbonate 12. Compound 13 was then converted to methyl 2-deoxy-2-dideuterio-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (13 → 14 → 15 → 16 → 17) in high overal yields. Subsequently, thymine, cytosine, 6-chloropurine and 2-amino-6-chloropurine were glycosylated with the α-chlorosugar 17 to give the regiospecifically labelled 2'-deoxy-2',2''-dideuterio-β-D-nucleosides.

Nuclear Magnetic Resonance (NMR) spectroscopy has proved to be a very powerful tool for understanding the conformational and dynamic aspects of both single and double stranded DNA and their interactions with metal ion, drugs, intercalating agents and proteins[1-6]. Two-dimentional (2D) NMR studies[3,4] like J correlated spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY) and very recently, J independent Hartmann-Hahn spin-locked (HOHAHA) pulse sequences[5] have been particularly useful in the assignment of different sugar-proton absorptions from each pentofuranosyl sugar unit in a DNA molecule[1-6]. In order to understand both the handedness of a DNA molecule and the stacking ⇄ unstacking equilibrium within a DNA molecule, it is desirable that the full J network of each constituent sugar residues is assigned and spin-spin couplings retrieved, allowing performance of spin-spin simulation experiments. Such excercises allow one to estimate the phosphate backbone conformation and also the population of pseudorotamers[5] at a particular temperature, % N (3'-endo) versus %S (2'-endo), for each sugar residue which then can be used to understand the thermodynamics of stacking ⇄ unstacking in a DNA molecule. On the other hand, upon establishing the J network of each sugar residue by COSY and/or HOHAHA pulse sequence, one employs NOESY to establish the NOE between the base proton and its own sugar H-1' and the sugar H-1' in the 5'-direction and the sugar H-1' in the 3'-direction in order to establish the handedness of a DNA molecule. Despite all these exercises, it is still difficult to obtain information on the mobilities of each sugar unit even from a small oligonucleotide (10-16 bases). This is clearly because of severe spectral overlap and lack of adequate resolution of the sugar-proton resonances

and the difficulty of labelling sugars regio and stereospecifically with deuterium[8,9]. An examination of 1D or 2D ^{1}H-NMR spectra of an oligonucleotide immediately reveals[1-5] that it is the H-2' and H-2'' and H-5' and H-5'' region of the spectra which contains important information regarding the sugar and the phosphate backbone conformation and are least resolved and are most complex to analyze. We herein report a general approach to the synthesis of 2'-deoxy-2',2''- dideuterio-nucleosides providing a means for incremental analysis of H-2'/2'' region of oligonucleotides by ^{1}H-NMR spectroscopy by selective suppression of H-2'/H-2'' absorptions at particular sites of oligo-DNA molecule containing these nucleosides and observe the sugar mobility by ^{2}H-NMR spectroscopy[8]. Any synthetic procedure developed for the above purpose should be generally applicable to all four 2'-deoxynucleosides and also should employ inexpensive reagents and protective groups in order to be synthetically useful. We first considered the procedure developed by Robins and his coworkers[10] for selective oxidation of the 2'-hydroxyl function of 1a to the 2'-keto derivative 2a and then $NaBD_4$ reduction and F^- ion treatment to give 4a in ca. 60% yield after an ion exchange column chromatography. These workers, in an independent paper, have also developed a useful procedure[11] to convert the 2'-O-(S-phenoxythiocarbonate) to its 2'-deoxy nucleosides by reduction with Bu_3SnD in high yield. It was therefore considered logical to combine these two procedures to give the 2'-deoxy-2',2''-dideuterio-nucleosides. However, the execution of this plan faced a few drawbacks: (i) DMSO-Ac_2O promoted oxidation[10] of 1a-1d gave 7-10% of 2-methythio ethers 3a-3d; (ii) it became laborious and cumbersome to purify the 2'-ketonucleoside after the removal of DMSO; (iii) one had to employ a large excess of $NaBD_4$ (52 atomic equiv.)[10] for reduction since this was carried out directly without the removal of solvent and reagent (DMSO-Ac_2O); (iv) during the $NaBD_4$ reduction in DMSO, 3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxyl)- (TPDS) group in 1a-1d was not stable and, in fact, ca. 30% of 5a-5d could be only isolated and the rest was deprotected to 4a-4d which, therefore, had to be reprotected again[12] with the expensive TPDS-Cl to give 5. Thus, it became clear during above work[12] that one of the major problems was the oxidation step with DMSO-Ac_2O. We, therefore, attempted the oxidation of 1a-d by a CrO_3--pyridine-acetic anhydride complex in CH_2Cl_2 at 20°C[10,13] despite the reported problems[10] with such oxidizing agent. It turned out that the oxidation of appropriately protected uridine derivative 1b by CrO_3-pyridine-acetic anhydride complex in CH_2Cl_2 gave a clean Cr^{+3} free product 2b (78%) which could be unambiguously characterized by ^{1}H-NMR spectroscopy. However, a similar oxidation of appropriately protected adenosine 1a, N^4-acetyl cytidine 1c and guanosine derivative 1d gave oxidation product(s) whose purity or identity could not be established[10] neither chromatographically nor spectroscopically. Therefore, it was decided to reduce directly the putative oxidation product(s) 2a, 2c and 2d to the corresponding 2'-OH derivatives 5a-d. Pyrimidine nucleosides (2b and 2c) were reduced by $NaBD_4$ in absolute ethanol (one molar equiv.) to the corresponding alcohol in good overall yields (ca. 60% in two steps). It may be noted that the corresponding $LiAlD_4$ reduction of pyrimidine derivatives gave a mixture products. Purine nucleosides (2a and 2d) were however reduced by $LiAlD_4$ (equimolar) in dry THF. Adenosine derivative 1a gave 5a with an overall yield of 57% in two steps (1a → 2a → 5a) but the corresponding guanosine derivative (2d) gave a mixture of products, at the end of two-step oxidation-reduction procedure, which were not characterized. It is likely that CrO_3-pyridine-Ac_2O oxidation step promotes side reaction in the guanine part of the nucleoside. Appropriately protected arabinosides 5a, 5b and 5c were then converted to their 2'-O-phenoxythiocarbonyl-[11] derivatives 6a, 6b and 6c in 81, 77 and 77% yields respectively. They were then subjected to n-Bu_3SnD reduction in dry toluene in presence of azoisobutyronitrile (AIBN)[11], followed by a deprotection step with fluoride ion, and a brief NH_3/MeOH for 7c, gave free 2',2''-dideuterio-2'-deoxy nucleosides 7a, 7b and 7e in 66, 70 and 72% yields respectively.

Since above procedure does not work well for the preparation of 2'-deoxy-2',2''-dideuterio-guanosine, and moreover, the preparation of 2'-deoxy-2',2''-dideuterio thymidine would involve an additional sequence of reactions for C-5 methylation of either uridine or 2'-deoxy-2',2''-dideuteriouridine, we decided to devise a general synthetic methodology for the preparation of



1 (a - d) 2 (a - d) 5 (a - d)

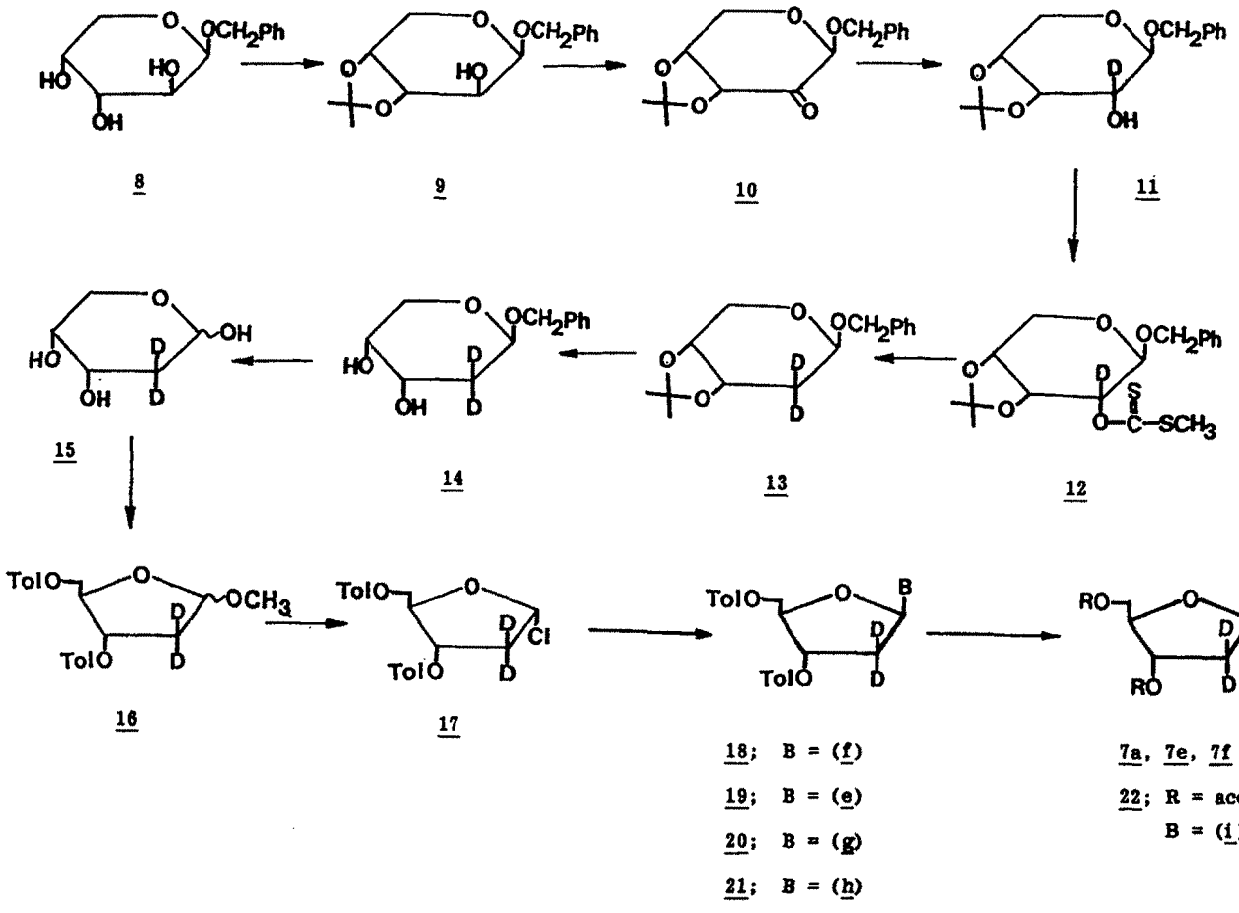
7 (a, b, c, e) 6 (a - c)

3 (a - d)

4 (a - d)

B = (a) 9-ADENINYL, (b) 1-URACILYL, (c) N^4-ACETYL-1-CYTOSINYL
(d) 9-GUANINYL, (e) 1-CYTOSINYL, (f) 1-THYMINYL, (g) 6-CHLORO-9-PURINYL,
(h) 2-AMINO-6-CHLORO-9-PURINYL, (i) N^2-ACETYL-9-GUANINYL.

2-deoxy-2-dideuterioribose, which then can be suitably coupled, through its chlorosugar, to different nucleobases to give all four naturally occurring 2'-deoxy-2',2''-dideuterionucleosides. For this purpose, we chose isomerically pure benzyl 6-D-arabinopyranoside[14] (8) as a cheap and readily accessible starting material because of problems of selectively protecting the C-3 and C-5 hydroxyl groups of a methyl pentofuranoside (ara or ribo). Compound 8 was converted to 3,4-isopropylidene derivative[15] 9 in 89% yield leaving the C-2 hydroxyl group free which was oxidized by chromium trioxide-pyridine-Ac_2O complex[11,13] in dichloromethane at room temperature to the 2-keto sugar 10 in 88% yield. Compound 10 was then conveniently reduced with $LiALD_4$ in dry THF preferentially to the crystalline 2(R)-deuterio-alcohol 11 in 94% yield. The second deuterium at the C-2 was then introduced by converting the 2(R)-deuterio-alcohol 11 to a crystalline 2-O-(S--methyldithiocarbonate) derivative[16] 12 in 97% yield followed by its reductive cleavage with Bu_3SnD in refluxing toluene for 20 h to give the 2-deoxy-2,2'-dideuterio sugar 13 in 79% yield. The 3,4-isopropylidene group in 13 could be selectively removed with 80% acetic acid at 20°C for 24 h to crystalline 14 in 80% yield and then to 2-deoxy-2,2'-dideuterioribose 15 in 93% yield upon treatment with 0.5 M aqueous hydrochloric acid for 36 h at 20°C. At this stage, the 2-deoxy-2,2'--dideuteriosugar was converted to its methyl 3,5-di-(p-toluoyl)furanoside (α/β-mixture) 16. The α/β-mixture of methyl glycosides 16 was then converted to isomerically pure crystalline 2-deoxy-2,-2'-dideuterio-3,5-di-O-(p-toluoyl)-D-erythropentofuranosyl chloride (17) in 83% yield using reported procedure for non-deuterated sugar[17].


8
9
10
11
12
13
14
15
16
17
18; B = (f)
19; B = (e)
20; B = (g)
21; B = (h)
7a, 7e, 7f (R = H)
22; R = acetyl
B = (i)

Synthesis of 2'-deoxy-2',2''-dideuterio nucleosides.

The 2-deoxy-2,2'-dideuterio-α-chloro sugar 17 was subsequently used for condensation reactions with thymine[9,18], 6-chloropurine[9,19] and 2-amino-6-chlorpurine[9] to give the corresponding 2'-deoxy-2',2''-dideuterio-nucleosides 18, 20 and 21 in 81, 55 and 51% yields respectively. The condensation of 17 and cytosine gave a mixture of 19 and its α-isomer which could not be separated in the protected form and, therefore, the mixture of 19 + α-isomer was directly deprotected using literature procedures[18] and separated on a Dowex-OH⁻ column to obtain pure 7e in 40% yield. Compounds 18 and 20 were also deprotected to free nucleosides 7f and 7a in 89 and 81% yields respectively. Compound 21 was also converted to the guanine-nucleoside by a treatment of trimethylamine in diglyme at 20°C for 2 h followed by a treatment with tetraethylammonium hydroxide for overnight at 20°C; however it was isolated as its triacetate 22.

The ^{1}H-NMR spectra of the 2'-deoxy-2',2''-dideuterionucleosides are shown in panel B while the corresponding non-deuterated (natural) nucleosides are shown in panel A in figures 1, 2, 3, 4 and 5. A comparison of these spectra in figures 1-5 clearly illustrate the specific labellings achieved in the present work. The spectra in Figs. 1-5 also clearly show that an absence of the J net work through H-2' and H-2'' in the pentose-sugar part has brought about a severe change in the J multiplicities of H-1', showing now a singlet, and H-3' which only show the expected vicinal coupling with the H-4'. Further work is in progress to show that the incorporation of these 2',2''-dideuterio-nucleosides along with the natural ones in oligo-DNA can indeed help to mask signals selectively in the H-2'/2'' region and this also masks multiplicities in the anomeric and H-3' region which can be used in turn, to retrieve full information from the nondeutrated sugars and then systematically altering the sites of incorporations of these deuterio-sugars within the same DNA molecule, one should be able to build up the total picture on conformation and dynamics of bigger DNA molecule in an incremental fashion.

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 was used as the internal standard and the chemical shifts are reported in ppm (δ scale). UV absorption spectra were recorded with a Varian-Cary 2200 instrument and Jeol DX 303 instrument was used for recording the mass spectra. IR absorption was recorded with Perkin-Elmer 298 spectrometer. Thin-layer chromatography (t.l.c.) was performed on Merck precoated $60F_{254}$ plates. Merck Kieselgel G was used for short column chromatography.

6-Amino-9-(3',5'-O-TPDS-2'(S)-deuterio-β-D-threo-pentofuranosyl)purine (5a).

Compound 1[11] (2.36 g, 4 mmol) was added to a solution of the pre-mixed complex of chromium trioxide(1)-pyridine(2)-acetic anhydride(1) (1.2 g - 2 ml - 1.2 ml, 3 molar equiv. to nucleoside) in dichloromethane (30 ml) and the mixture was stirred at room temperature for 50 min. The resulting dark brown solution was transferred by pipet into a silica gel column covered with ethyl acetate (150 ml). The column was then eluated with ethylacetate. Solvent was evaporated and coevaporated with toluene to dryness. The residue was dissolved in dry tetrahydrofuran (THF) (50 ml). The solution was cooled with ice-water bath and lithium aluminium deuteride (98 atom % D, 168 mg, 4 mmol) was added. Stirring was continued for 2 h and then water (1 ml) was added into the mixture to destroy the excessive lithium aluminium deuteride. Centrifugation, evaporation and purification of the mixture with silica gel column gave 5a, yield 1.35 g (57%). MS (FAB$^+$): calc. 511. 2631 for $(M+H)^+$, found 511.2617. ^{1}H-NMR ($CDCl_3$): 8.13 (s, 2H) H-8, H-2; 6.49 (broad, 2H) $-NH_2$; 6.21 (s, 1H) H-1'; 4.59 (d, $J_{3',4'}$ = 8.1 Hz, 1H) H-3'; 4.03-3.62 (m, 4H) H-4', H-5' and -OH; 1.08 (m, 28H) isopropyl groups. ^{13}C-NMR ($CDCl_3$): 83.7 (d, J_{CH} = 166 Hz) C-1'; 81.1 (d, J_{CH} = 145.3 Hz) C-4'; 74.4 (d, J_{CH} = 144.2 Hz) C-3'; 61.4 (t, J_{CH} = 141.6 Hz) C-5'.

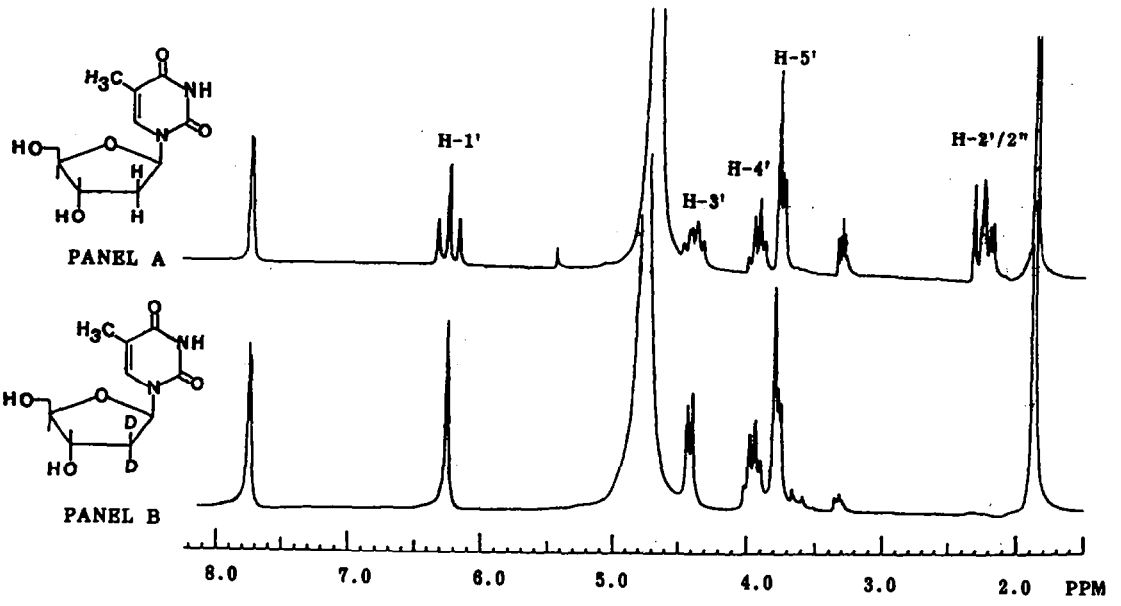


FIGURE 1

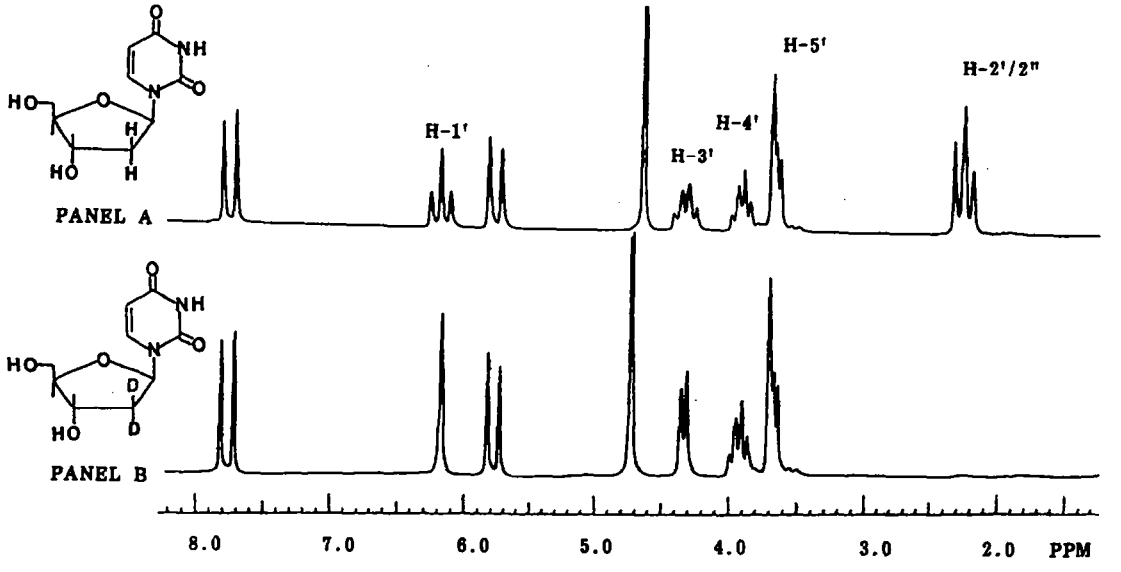


FIGURE 2

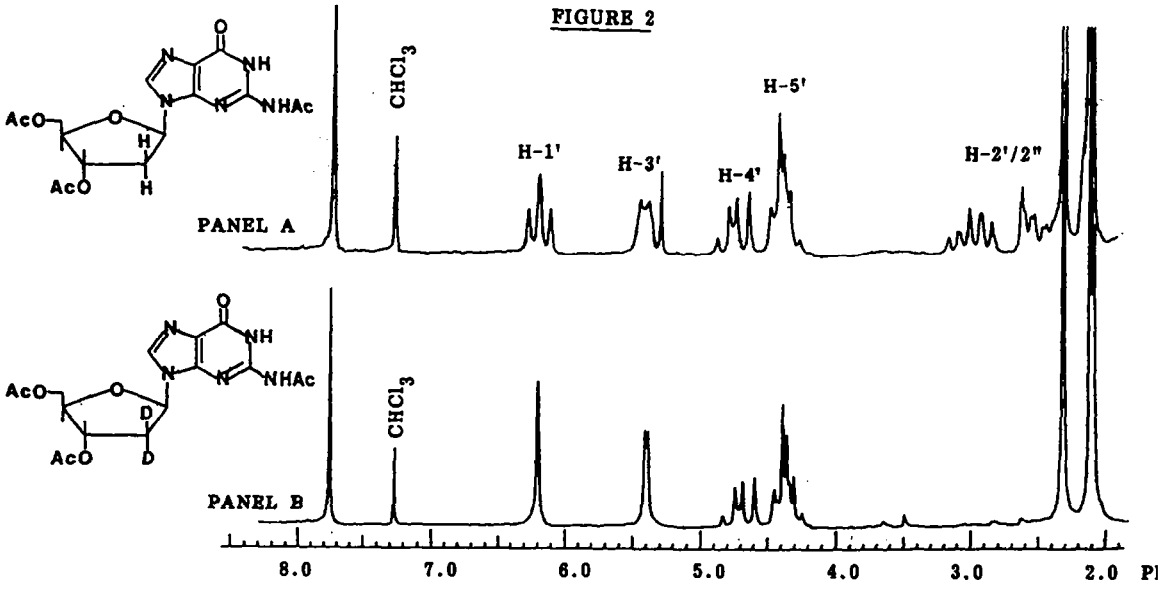


FIGURE 3

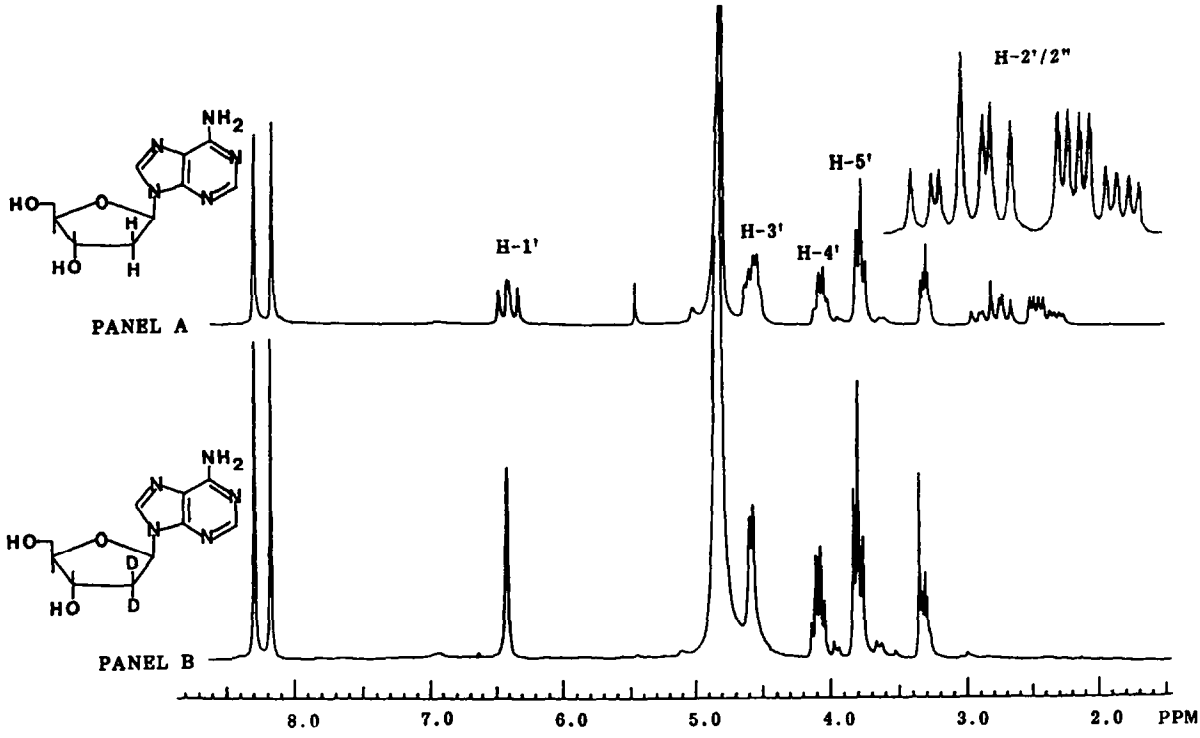


FIGURE 4

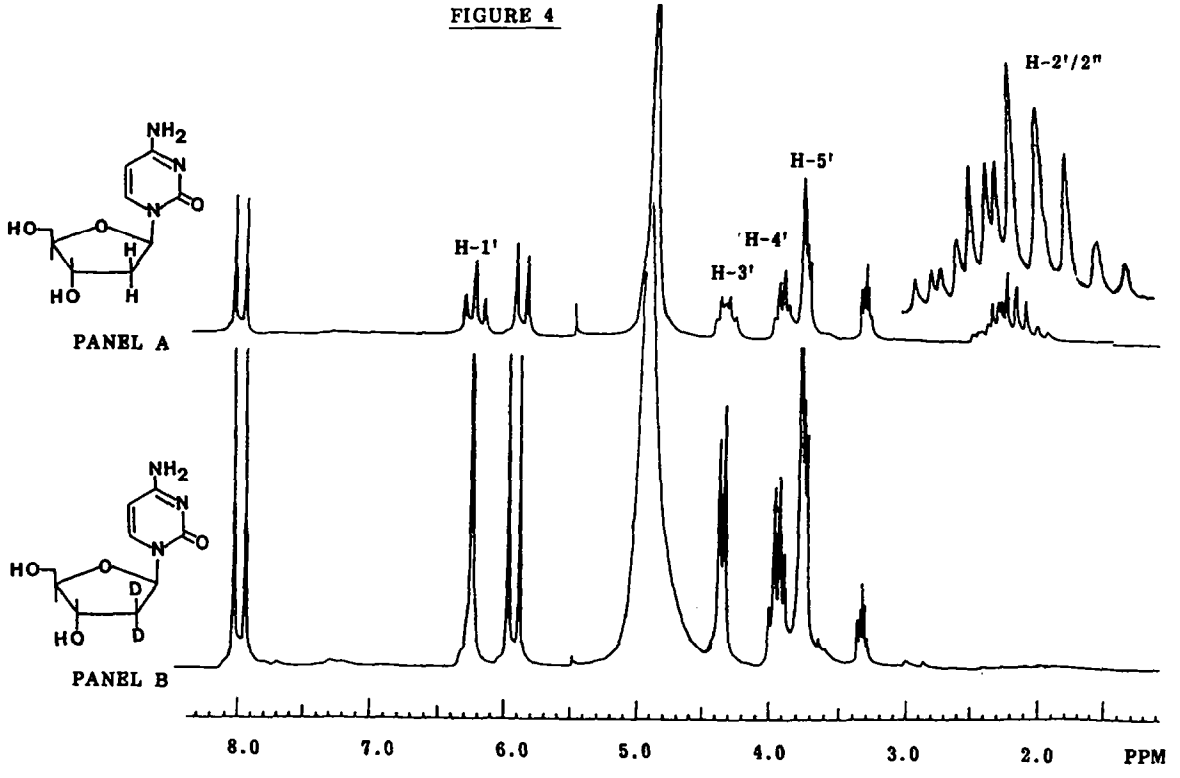


FIGURE 5

6-Amino-9-(3',5'-O-TPDS-2'-O-phenoxythiocarbonyl-2'(S)-deuterio-β-D-threo-pentofuranosyl)purine (6a).

The mixture of 5a (1.1 g, 2.16 mmol), 4-N,N-dimethylaminopyridine (DMAP) (0.54 g, 4.43 mmol), phenoxythiocarbonyl chloride (PTC-Cl) (471 µl, 2.59 mmol) in dry acetonitrile (35 ml) was stirred at room temperature overnight. Solvent was evaporated and the residue was partitioned between ethyl acetate (100 ml) and water (50 ml). Organic phase was washed with water (2 x 50 ml), dried with

$MgSO_4$, evaporated. The residue was purified with a silica gel column to 6a, yield 1.06 g (81%). MS (FAB^+): calc. 647.2614 for $(M+H)^+$, found 647.2603. ^{1}H-NMR ($CDCl_3$): 8.32 (s, 1H) H-8; 7.98 (s, 1H) H-2; 7.23-6.75 (m, 5H) arom; 6.20 (s, 1H) H-1'; 5.21 (d, $J_{3',4'}$ = 8.1 Hz, 1H) H-3'; 4.16-4.06 (m, 3H) H-4', H-5'; 1.08 (m, 28H) isopropyl groups. ^{13}C-NMR ($CDCl_3$): 80.5 (d, J_{CH} = 170.8 Hz) C-1'; 80.5 (d, J_{CH} = 146.4 Hz) C-4'; 73.1 (d, J_{CH} = 145.3 Hz) C-3'; 61.7 (t, J_{CH} = 144.0 Hz) C-5'.

6-Amino-9-(2'-deoxy-2',2''-dideuterio-β-D-erythro-pentofuranosyl)purine (7a).

Compound 6a (0.91 g, 1.5 mmol) was dissolved in dry toluene (25 ml) and 2,2'-azo-bis-2-methyl- propanitrile (AIBN) (48 mg, 0.3 mmol) and n-Bu_3SnD (800 µl, 3 mmol) were added. The solution was degassed with nitrogen for 20 min and then heated at 75 °C under nitrogen for 3 h. To this mixture tetra-n-butylammonium fluoride (TBAF) (3 ml, 1 M in THF) was added and stirred at 75 °C for another 1 h. All volatile material was evaporated and the residue was partitioned between ether (5 x 30 ml) and water (50 ml). The aqueous phase was concentrated and applied to a column of Dowex 1-X2(OH^-) resin using water as the eluent. Appropriate fractions were collected and evaporated. The product was crystallized from ether-ethanol mixture to give 7a, yield 250 mg (66%). ^{1}H-NMR (CD_3OD + D_2O): 8.31 (s, 1H) H-8; 8.19 (s, 1H) H-2; 6.44 (s, 1H) H-1'; 4.60 (d, 1H, $J_{3',4'}$ = 2.4 Hz) H-3'; 4.10 (m, 1H, $J_{4',5'}$ = 3.4 Hz, $J_{4',5''}$ = 2.9 Hz) H-4'; 3.82 (m, 2H, $J_{5',5''}$ = 12.2 Hz) H-5' and H-5''. ^{13}C-NMR (CD_3OD + D_2O): 89.8 (d, J_{CH} = 148.9 Hz) C-1'; 87.0 (d, J_{CH} = 167.3 Hz) C-4'; 72.9 (d, J_{CH} = 142.8 Hz) C-3'; 63.6 (t, J_{CH} = 142.8 Hz) C-5'. MS (EI^+): calc. for M^+ 253.1144, found 253.1139.

1-(3',5'-O-TPDS-2'(S)-deuterio-β-D-threo-pentofuranosyl) 1H,3H-pyrimidine-2,4-dione (5b).

Compound 2b[11] (0.94 g, 1.93 mmol) was dissolved in absolute ethanol (10 ml) and was cooled with ice- water bath. Sodium borodeuteride (51 mg, 1.23 mmol) was added and stirring was continued for 2 h. The solution was neutralized by bubbling carbon dioxide. Solvent was evaporated and the residue was partitioned between ethyl acetate (100 ml) and water (50 ml). The organic phase was washed with saturated aqueous sodium chloride, dried with $MgSO_4$, evaporated to give 5b, yield 907 mg (96%). MS (FAB^+): calc. 488. 2359 for $(M+H)^+$, found 488. 2384. ^{1}H-NMR ($CDCl_3$): 7.84 (d, $J_{5,6}$ = 7.9 Hz, 1H) H-6; 6.10 (s, 1H) H-1'; 5.72 (d, 1H) H-5; 4.1-3.8 (m, 5H) H-3', H-4', H-5', -OH; 1.05 (m, 28H) isopropyl groups. ^{13}C-NMR ($CDCl_3$): 84.2 (d, J_{CH} = 173.3 Hz) C-1'; 80.7 (d, J_{CH} = 147.7 Hz) C-4'; 72.1 (d, J_{CH} = 136.7 Hz) C-3'; 60.2 (t, J_{CH} = 144.1 Hz) C-5'.

1-(3',5'-O-TPDS-2'-O-phenoxythiocarbonyl-2'(S)-deuterio-β-D-threo-pentofuranosyl)-1H,3H-pyrimidine-2,4-dione (6b).

Compound 5b (790 mg, 1.27 mmol) was treated under the same condition as described for 6a to give 6b, yield 780 mg (77%). MS (FAB^+): calc. 624. 2341 for $(M+H)^+$, found 624. 2373. ^{1}H-NMR ($CDCl_3$): 7.61 (d, $J_{5,6}$ = 8.3 Hz, 1H) H-6; 7.3-7.0 (m, 5H) arom; 6.38 (s, 1H) H-1'; 5.76 (d, 1H) H-5; 4.63 (d, $J_{3',4'}$ = 8.5 Hz, 1H) H-3'; 4.10 (m, 2H) H-5'; 3.90 (m, 1H) H-4'; 1.07 (m, 28H) isopropyl groups. ^{13}C-NMR ($CDCl_3$): 81.2 (d, J_{CH} = 177 Hz) C-1'; 80.5 (d, J_{CH} = 162 Hz) C-4'; 72.1 (d, J_{CH} = 150 Hz) C-3'; 60.5 (t, J_{CH} = 144 Hz) C-5'.

1-(2'-deoxy-2',2''-dideuterio-β-D-erythro-pentofuranosyl)-1H,3H-pyrimidine-2,4-dione (7b).

Compound 6b (623 mg, 1 mmol) was dissolved in dry toluene (20 ml) and AIBN (32 mg, 0.2 mmol) and Bu_3SnD (523 µl, 2 mmol) were added. The solution was degassed with nitrogen for 20 min and then heated at 75 °C for 3 h. TBAF (2 ml, 1 M in THF) was added and the mixture was stirred for another 1 h at 75 °C. Solvent was evaporated and the residue was partitioned between ether (50 ml) and water (50 ml). The aqueous phase was concentrated and applied to a column of Dowex 1-X2 (HCO_3) resin. elution of product with water to give 7b, yield 170 mg (75%). MS (FAB^-):

calc. 229. 0794 for $(M-H)^-$, found 229. 0769. ^{1}H-NMR (D_2O): 7.78 (d, $J_{5,6}$ = 8.1 Hz, 1H) H-6; 6.20 (s, 1H) H-1'; 5.81 (d, 1H) H-5; 4.38 (d, $J_{3',4'}$ = 3.4 Hz, 1H) H-3'; 3.97 (m, 1H) H-4'; 3.74 (m, 2H) H-5'. ^{13}C-NMR (D_2O): 88.2 (d, J_{CH} = 149.0 Hz) C-4'; 85.9 (d, J_{CH} = 177 Hz) C-1'; 71.9 (d, J_{CH} = 155 Hz) C-3'; 62.7 (t, J_{CH} = 149 Hz) C-5'.

4-Acetamido-1-(3',5'-O-TPDS-2'(S)-deuterio-β-D-threo-pentofuranosyl)-1H-pyrimidine-2-one (5c).

Oxidation of compound 1c[20] (527 mg, 1 mmol) was carried out under the same condition as described for 1b. The crude ketonucleoside was dissolved in absolute ethanol (4 ml), ammonium chloride (54 mg, 1 mmol) and sodium borodeuteride (42 mg, 1 mmol) were added at 0 °C. Stirring was continued for 2 h and then methanol (1 ml), saturated aqueous ammonia chloride (2 ml) were added. After evaporation of the solvent the residue was partitioned between ethyl acetate (50 ml) and water (20 ml). the organic phase was washed with saturated aqueous sodium chloride (2 x 20 ml), dried with $MgSO_4$, evaporated. The mixture was separated with silica gel column to give 5c, yield 300 mg (57%). MS (FAB^+): calc. 529. 2625 for $(M+H)^+$, found 529. 2604. ^{1}H-NMR ($CDCl_3$): 8.23 (d, $J_{5,6}$ = 7.5 Hz, 1H) H-6; 7.51 (broad, 1H) H-5; 6.14 (s, 1H) H-1'; 4.13-3.81 (m, 5H) H-3', H-4', H-5', -OH; 2.27 (s, 3H) acetyl; 1.09 (m, 28H) isopropyl group. ^{13}C-NMR ($CDCl_3$): 85.2 (d, J_{CH} = 173.4 Hz) C-1'; 81.0 (d, J_{CH} = 145.7 Hz) C-4'; 73.3 (d, J_{CH} = 141.6 Hz) C-3'; 60.5 (t, J_{CH} = 145.3 Hz) C-5'; 24.3 CH_3 in acetyl.

4-Acetamido-1-(3',5'-O-TPDS-2'-O-phenoxythiocarbonyl-2'(S)-deuterio-β-D-threo-pentofuranosyl)-1H-pyrimidine-2-one (6c).

The mixture of 5c (370 mg, 0.2 mmol), DMAP (769 mg, 6.3 mmol) and PTC-Cl (172.5 mg, 1.4 mmol) in dry acetonitrile (10 ml) was stirred at 20 °C for 24 h. Solvent was evaporated and the residue was partitioned between ethyl acetate (3 x 100 ml) and water (50 ml). The organic phase was washed with satd. aqueous sodium chloride (2 x 30 ml), dried with $MgSO_4$, evaporated. The residue was purified with silica gel column to give 6c, yield 350 mg (77%). MS (FAB^+): calc. 665. 2607 for $(M+H)^+$, found 665. 2607. ^{1}H-NMR ($CDCl_3$): 8.0 (d, $J_{5,6}$ = 7.1 Hz, 1H) H-6; 7.27-6.95 (m, 6H) H-5, arom; 6.43 (s, 1H) H-1'; 4.61 (d, $J_{3',4'}$ = 7.5 Hz, 1H) H-3'; 4.1-3.96 (m, 3H) H-4', H-5'; 2.28 (s, 3H) acetyl; 1.12 (m, 28H) isopropyl groups. ^{13}C-NMR ($CDCl_3$): 82.9 C-1'; 81.4 C-4'; 72.4 (d, J_{CH} = 141.7 Hz) C-3'; 60.9 (t, J_{CH} = 138.1 Hz) C-5'; 25.0 acetate CH_3.

4-Amino-1-(2'-deoxy-2',2''-dideuterio-β-D-erythro-pentofuranosyl)-1H-pyrimidine-2-one (7e).

Compound 6c (305 mg, 0.46 mmol) was dissolved in dry toluene (8 ml) and AIBN (16 mg, 0.1 mmol) and Bu_3SnD (250 µl, 0.92 mmol) were added. The solution was degassed with nitrogen for 20 min and the mixture was heated at 75 °C for 14 h under nitrogen. TBAF (1 ml, 1 M in THF) was added and stirring was continued for 3 h. Solvent was evaporated and the residue was treated with saturated ammonia-methanol overnight. All volatile matters were evaporated and the residue was partitioned between ether (20 ml) and water (20 ml). The aqueous phase was applied to a column of Dowex 1-X2 (OH^-) resin. Elution with water and evaporation of appropriate fractions gave 7e, yield 40 mg (38%). ^{1}H-NMR (CD_3OD + D_2O): 7.98 (d, $J_{5,6}$ = 7.5 Hz, 1H) H-6; 6.25 (s, 1H) H-1'; 5.94 (d, 1H) H-5; 4.38 (d, $J_{3',4'}$ = 3.9 Hz, 1H) H-3'; 3.96 (m, $J_{4',5'}$ 4 3.4 Hz, $J_{4',5''}$ = 3.9 Hz, 1H) H-4'; 3.79 (m, 2H) H-5' and H-5''. ^{13}C-NMR (CD_3OD + D_2O): 88.7 (d, J_{CH} = 147 Hz) C-4'; 87.6 (d, J_{CH} = 170 Hz) C-1'; 72.1 (d, J_{CH} = 150.2 Hz) C-3'; 62.9 (t, J_{CH} = 141.6 Hz) C-5'. MS (EI^+) calc. for M^+ 229.1031, found 229.1026.

Benzyl 3,4-isopropylidene-β-D-arabino-pentopyranoside (9).

To a suspension of benzyl β-D-arabinopyranoside (8) (13 g, 57 mmol) in DMF (100 ml), P-toluen- sulfonic acid monohydrate (140 mg, 0.74 mmol) and 2,2-dimethoxypropane (21.3 ml, 171 mmol) were added. The mixture was stirred 2 h at 20 °C. the reaction was neutralized with Amberlite (OH^- form). The mixture was filtered and the filtrate was evaporated and co-evaporated a few times with toluene. The residue was then purified by silica gel column chromatography to give the title compound 9, wh-

ich was precipitated from petroleum ether. Yield 13.5 g (89%). mp. 90-92 °C. ^{1}H-NMR ($CDCl_3$): 7.36 (s, 5H) arom.; 4.93 (d, $J_{1,2}$ = 3.6 Hz, 1H) H-1; 4.68 (q, J_{gem} = 12.4 Hz, 2H) O-CH_2Ph; 4.23 (m, 2H) H-3 and H-4; 3.95 (s, 2H) H-5; 3.82 (broad s, 1H) H-2; 2.35 (broad d, 1H) -OH; 1.53 and 1.36 (2 x s, 6H) CH_3 in isopropylidene. ^{13}C-NMR ($CDCl_3$): 96.7 (C-1); 75.7 (C-2); 72.7 (C-3); 69.9 (C-4); 69.6 (C-5); 59.7 (-OCH_2Ph); 27.8 and 25.8 (CH_3 in isopropylidene).

Benzyl 3,4-isopropylidene-β-D-erythro-pentopyranosid-2-ulose (10).

Chromium (VI) oxide (5.96 g, 59.7 mmol) was added to pyridine (12 ml, 119.4 mmol) in dichloromethane (100 ml) and the resulting solution was stirred for 15 min at 20°C. A solution of 9 (4 g, 14.9 mmol) in dichloromethane (10 ml) was added, immediately followed by acetic anhydride (5.8 ml, 59.7 mmol). After stirring for 15 min, ethyl acetate (50 ml) and toluene (50 ml) were added. The solution was decanted and passed through a short silica gel column. The eluate was evaporated and co-evaporated with toluene to give 10 as an oil (3.52 g, 88%). ^{1}H-NMR ($CDCl_3$): 7.32 (s, 5H) arom.; 4.88 (s, 1H) H-1; 4.67 (q, J_{gem} = 12 Hz, 2H) OCH_2Ph; 4.70 (d, $J_{3,4}$ = 5.1 Hz, 1H) H-3; 4.50 (m, 1H) H-4; 4.22 (m, 2H) H-5; 1.44 and 1.37 (2 x s, 6H) CH_3 of isopropylidene. ^{13}C-NMR ($CDCl_3$): 198.5 (s) carbonyl group; 98.7 (d, J_{CH} = 174.1 Hz) C-1; 77.3 (d, J_{CH} = 149.4 Hz) C-3; 75.1 (d, J_{CH} = 115.7 Hz) C-4; 69.7 (t, J_{CH} = 107.8) C-5; 58.3 (t, J_{CH} = 144.9 Hz) O-CH_2-Ph; 26.9 and 25.8 methyl in isopropylidene. IR = 1750 cm^{-1} (C=O). MS (FAB$^-$): calc. for (M-H) 277.1076, found 277.1094.

Benzyl-3,4-isopropylidene-2(R)-deuterio-β-D-erythro-pentopyranoside (11).

Compound 10 (13.24 g, 47.6 mmol) in dry THF (100 ml) solution was cooled in a ice-water bath and lithium aluminium deuteride (98% atom D, 0.98 g, 23.3 mmol) was added in portions. The mixture was stirred 15 min in the ice-water bath and then at room temperature for 3 h until the disappearence of carbonyl band absorption was observed in the IR spectra. The reaction was quenched with water (1 ml) and then ethyl acetate (50 ml) was added. The mixture was filtered and the filtrate was evaporated to a small volume (15 ml). Ethyl acetate (150 ml) was added and then washed with water (2 x 15 ml). Organic phase was dried ($MgSO_4$) and evaporated to give 11 which was crystallized from petroleum ether. Yield 12.5 g (94%). mp. 90-2°C (methanol). $C_{15}H_{19}DO_5$ requires: C 64.04, H/D 7.52; found C 65.11, H/D 7.59. ^{1}H-NMR ($CDCl_3$): 7.34 (s, 5H) arom.; 4.83 (s, 1H) H-1; 4.67 (q, J_{gem} =12 Hz, 2H) OCH_2Ph; 4.45 (d, $J_{3,4}$ = 6.7 Hz, 1H) H-3; 4.24 (m, 1H) H-4; 3.76 (m, 2H, $J_{4,5}$ = 3 Hz, J_{gem} = 12 Hz) H-5; 2.64 (broad s, 1H) -OH; 1.53 and 1.36 (2xs, 6H) CH_3 in isopropylidene; ^{13}C-NMR ($CDCl_3$): 99.3 (d, J_{CH} = 161.8 Hz) C-1; 72.7 (d, J_{CH} = 150.4 Hz) C-3; 72.6 (d, J_{CH} = 150.4 Hz) C-4; 69.7 (t, J_{CH} = 132.4 Hz) C-5; 61.7 (t, J_{CH} = 147.0 Hz) OCH_2Ph; 26.3 and 25.1 CH_3 of isopropylidene. MS (FAB$^-$): calc. for (M-H)$^-$ 280.1295, found 280.1313.

Benzyl 3,4-isopropylidene-2(R)-deuterio-2-O-[(methylthio)thiocarbonyl]-β-D-erythro-pentopyranoside (12).

A solution of 11 (5.34 g, 19 mmol) in THF (100 ml) was added to a stirring suspension of NaH (1.2 g, 80% in oil, 40 mmol) in THF at 0 °C. The mixture was refluxed 2 h and then cooled in ice-water bath. Carbon disulfide (10 ml) was added slowly with vigorous stirring. Stirring was continued for further 2 h at room temperature. Methyl iodide (10 ml, 39.9 mmol) was added and the mixture was kept overnight. The solution was poured into ice-water with stirring and extracted with ethylacetate (3 x 100 ml). Organic phase was washed with water, dried ($MgSO_4$) and evaporated to give 6.85 g (97%) yellowish solid 12. mp. 82-4 °C (methanol). $C_{17}H_{21}D_1O_5S_2$ requires: C 54.96, H/D 6.24; found C 55.11, H/D 6.31. ^{1}H-NMR ($CDCl_3$): 7.31 (s, 5H) arom.; 5.07 (s, 1H) H-1; 4.78 (d, $J_{3,4}$ = 7.1 Hz, 1H) H-3; 4.69 (q, J_{gem} = 12 Hz, 2H) OCH_2Ph; 4.38 (m, $J_{4,5}$ = 1.9 Hz, $J_{4,5'}$ = 2.1 Hz, 1H) H-4; 3.82 (m, J_{gem} = 12.2 Hz, 2H) H-5; 2.57 (s, 3H) -SCH_3; 1.52 and 1.32 (2xs, 6H) CH_3 of isopropylidene. ^{13}C-NMR ($CDCl_3$): 96.1 (d, J_{CH} = 164.0 Hz) C-1; 73.2 (d, J_{CH} = 150.9 Hz) C-3; 70.7 (d, J_{CH} = 159.5 Hz) C-4; 69.2 (t,

J_{CH} = 147.1 Hz) C-5; 62.2 (t, J_{CH} = 148.3 Hz) $O\underline{CH_2}Ph$; 26.2 and 25.1 (q each) CH_3 of isopropylidene; 19.2 (q, J_{CH} = 142.7 Hz) -$\underline{SCH_3}$; MS (EI^+): calc. for M^+ 371.0972 found 371.0979.

Benzyl 3,4-isopropylidene-2-deoxy-2,2'-dideuterio-erythro-pentopyranoside (13).

To a boiling solution of tributyltin deuteride (4.7 g, 15 mmol) in dry toluene (150 ml) was added dropwise over a period of 3 h, a solution of 12 (3.7 g, 10 mmol) in dry toluene (100 ml) under argon which was refluxed for 20 h while the reaction was monitored by t.l.c. (10:1, v/v; Benzene-ethyl acetate). The solvent was removed in vacuo and the residue was extracted with hot acetonitrile (3 x 80 ml). The extract was washed with hexane (4 x 50 ml) to remove tin-containing compounds. After concentration of the acetonitrile layer, the residue was crystallized from hexane. The mother liquor was purified by silica gel column chromatography to give the title compound. Yield: 2.1 g, 79%. mp. 48-9 °C (hexane). $C_{15}H_{18}D_2O_4$ requires: C 67.65, H/D 8.32; found C 67.51, H/D 8.39. ^{1}H-NMR ($CDCl_3$): 7.32 (s, 5H) arom.; 4.97 (s, 1H) H-1; 4.62 (q, J_{gem} = 11.8 Hz, 2H) $O\underline{CH_2}Ph$; 4.46 (d, $J_{3,4}$ = 6.3 Hz, 1H) H-3; 4.12 (m, $J_{4,5}$ = $J_{4,5'}$ = 2.7 Hz, 1H) H-4; 3.79 (m, J_{gem} = 12.1 Hz, 2H) H-5; 1.50 and 1.34 (2xs, 6H) CH_3 of isopropylidene. ^{13}C-NMR ($CDCl_3$): 95.5 (d, J_{CH} = 170.0 Hz) C-1; 71.9 (d, J_{CH} = 102.5 Hz) C-4; 69.6 (d, J_{CH} = 99.5 Hz) C-3; 69.1 (t, J_{CH} = 148.7 Hz) C-5; 61.1 (t, J_{CH} = 145.3 Hz) $O\underline{CH_2}Ph$; 27.2 and 25.3 CH_3 in isopropylidene. MS (EI^+): calc. for M^+ 266.1487, found 266.1466.

Benzyl 2-deoxy-2,2'-dideuterio-β-D-ribopyranoside (14).

Compound 13 (3.7 g, 13.9 mmol) was dissolved in 80% acetic acid (30 ml) and the solution was stirred at room temperature for 24 h and then evaporated to dryness. The residue was subsequently crystallized from ether. Yield 2.5 g, 80%. mp. 96-7 °C (ether). $C_{12}H_{14}D_2O_4$ requires: C 63.70, H/D 8.02; found 63.78, H/D 8.11. ^{1}H-NMR ($CDCl_3$): 7.30 (s, 5H) arom.; 4.95 (s, 1H) H-1; 4.53 (q, J_{gem} = 11.5 Hz, 2H) $O\underline{CH_2}Ph$; 4.09 (m, 2H) H-3 and H-4; 3.81 (m, 2H) H-5; ^{13}C-NMR ($CDCl_3$): 96.7 (d, J_{CH} = 170.0 Hz) C-1; 68.9 (t, J_{CH} = 140.1 Hz) C-5; 67.9 (d, J_{CH} = 100.0 Hz) C-4; 64.6 (d, J_{CH} = 142.7 Hz) C-3; 62.6 (t, J_{CH} = 147.8 Hz) $O\underline{CH_2}Ph$. MS (FAB^-): calc. for $(M-H)^-$ 225.1096, found 225.1072.

2-deoxy-2,2'-dideuterio-D-erythropentose (15)

The compound 14 (3.7 g, 16.4 mmol) was treated with hydrogen chloride solution (30 ml, 0.5 M) stirred at room temperature for 36 h. The reaction was neutralized with amberlite (OH^- form) filtered and the filtrate was washed with dichloromethane (2 x 20 ml) and ether (20 ml). Aqueous phase was evaporated in vacuo to give 1·4 g (93%) of an oil. MS (FAB^-) calc. for $(M-H)^-$ 135.0626, found 135.0629.

Methyl 2-deoxy-2,2'-dideuterio-3,5-di-O-p-toluoyl-D-erythro-pentofuranoside (16).

To a solution of compound 15 (0.6 g, 4.4 mmol) in methanol (12 ml) was added 1% solution of hydrogen chloride in methanol (1.3 ml). The mixture was kept in a stoppered flask for 15 min and then the reaction was stopped by adding excess of dry pyridine with stirring. The reaction mixture was evaporated and co-evaporated with dry pyridine and then the residue was dissolved in dry pyridine (4.6 ml). The solution was cooled in ice bath and p-toluoyl chloride was added quickly. The mixture was stirred at 0°C for 1 h and then stirred overnight at 20°C. The mixture was subsequently poured into crushed ice (20 ml) with stirring and extracted with ether (3 x 15 ml). Organic extracts were combined and washed with saturated sodium hydrogen carbonate (2 x 10 ml) and water (10 ml). The oranic phase was evaporated and purified by silica gel column chromatography. The title compound 16 was crystallized from ethanol. Yield 0.96 g (53%). ^{1}H-NMR ($CDCl_3$): 7.95-7.22 (m, 10H) arom.;

5.59 (d, $J_{3,4}$ = 2.5 Hz, 0.4H) H-3 of β-isomer; 5.41 (d, $J_{3,4}$ = 3.5 Hz, 0.6H) H-3 of α-isomer; 5.21 (s, 0.4H) H-1 of β-isomer; 5.19 (s, 0.6H) H-1 of α-isomer; 4.58 - 4.51 (m, 3H) H-4 and H-5 of α- and β-isomers; 3.41 (s, 1.8H) OCH_3 of α-isomer; 3.34 (s, 1.2H) OCH_3 of β-isomer; 2.40 (s, 6H) CH_3 on arom.; ^{13}C-NMR ($CDCl_3$): 105.4 (d, J_{CH} = 170.6 Hz) C-1 of β-isomer; 104.8 (d, J_{CH} = 170.7 Hz) C-1 of α-isomer; 81.7 (d, J_{CH} = 147 Hz) C-4 of β-isomer; 80.8 (d, J_{CH} = 149 Hz) C-4 of α-isomer; 75.2 (d, J_{CH} = 154 Hz) C-3 of β isomer; 74.4 (d, J_{CH} = 153 Hz) C-3 of α-isomer; 65.0 (t, J_{CH} = 150.5 Hz) C-5 of β-isomer; 64.1 (t, J_{CH} = 148 Hz) C-5 of α-isomer; 55.0 (q, J_{CH} = 143.8 Hz) OCH_3 α and β; MS (FAB$^-$): calc. for $(M-H)^-$ 385.1620, found 385.1630.

2-deoxy-2,2'-dideuterio-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride (17).

Compound 16 (0.96 g, 2.49 mmol) was dissolved in glacial acetic acid (1.4 ml) and to this solution was added glacial acetic acid which was presaturated with dry hydrogen chloride (3 ml). Dry hydrogen chloride gas was then bubbled into the solution when the product started crystallizing out. The reaction mixture was then cooled in ice-bath for 30 min and then the crystals were filtered and washed throughly with cold dry ether to give 17. Yield 0.81 g (83.5%). mp. 119-21 °C. $C_{21}H_{19}D_2ClO_5$ requires: C 64.53, H/D 5.93, Cl 9.07; found C 64.49, H/D 6.01, Cl 9.14. ^{1}H-NMR ($CDCl_3$): 7.90 (m, 4H) and 7.2 (m, 4H) arom.; 6.46 (s, 1H) H-1; 5.55 (d, $J_{3,4}$ = 2.6 Hz, 1H) H-3; 4.84 (m, 1H) H-4; 4.66 (m, 2H) H-5; 2.41 (s, 6H) CH_3 of arom.; ^{13}C-NMR ($CDCl_3$): 95.2 (d, J_{CH} = 187.6 Hz) C-1; 84.7 (d, J_{CH} = 148.3 Hz) C-4; 73.5 (d, J_{CH} = 156.7 Hz) C-3; 63.5 (t, J_{CH} = 147.2 Hz) C-5. MS (EI$^+$): calc. for $(M-Cl)^+$ 355.1515, found 355.1511.

1-(2'-Deoxy-2',2''-dideuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione (18).

A suspension of thymine (250 mg, 2 mmol) in a mixture of hexamethyldisilazane (4 ml) and trimethylchlorosilane (0.4 ml) was refluxed until it turned into a clean solution. Volatile matters was removed in vacuo and co-evaporated with dry xylene. The syrup was dissolved in pure chloroform (5 ml) and compound 17 (391 mg, 1 mmol) was added. The mixture was stirred for 24 h at room temperature. Chloroform (30 ml) was added and the solution was washed with water (3 x 10 ml). Organic layer was concentrated in vacuo and the residue was purified by silica gel column chromatography to give a mixture of α and β isomers which, upon dissolution in methanol, gave the crystals of the β isomer. Yield 390 mg (81.5%). mp. 196-7°C (methanol). $C_{26}H_{24}D_2N_2O_7$ requires: C 64.99, H/D 5.87, N 5.83; found C 65.12, H/D 5.94, N 5.91. ^{1}H-NMR ($CDCl_3$): 8.97 (s, 1H) -NH; 7.93 (m, 4H) arom.; 7.25 (m, 5H) arom. and H-6; 6.45 (s, 1H) H-1'; 5.62 (d, $J_{3,4}$ = 1.7 Hz, 1H) H-3'; 4.72 (m, 2H) H-5' and H-5''; 4.52 (m, 1H) H-4'; 2.43 (s, 6H) CH_3 on arom.; 1.62 (s, 3H) 5-CH_3; ^{13}C-NMR ($CDCl_3$): 84.9 (d, J_{CH} = 171.8 Hz) C-1'; 82.8 (d, J_{CH} = 151.6 Hz) C-4'; 74.8 (d, J_{CH} = 160 Hz) C-3'; 64.2 (t, J_{CH} = 146.5 Hz) C-5'. MS (FAB$^-$): calc. for $(M-H)^-$ 479.1787, found 479.1801.

1-(2'-Deoxy-2',2''-dideuterio-β-D-erythro-pentofuranosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione (7f).

Compound 18 (230 mg, 0.48 mmol) was dissolved in methanolic ammonia (25 ml) and the solution was stirred overnight. Volatile materials was removed in vacuo. The residue was dissolved in water (20 ml) and extracted with dichloromethane (2 x 15 ml) and ether (15 ml). Aqueous phase was concentrated to dryness to give 7f. Yield 104 mg (89%). mp. 181-2°C (methanol. UV (water): λ_{max} = 266 nm (ε = 9.300). $C_{10}H_{12}D_2N_2O_5$ requires: C 49.17, H/D 6.60, N 11.47; found C 49.11, H/D 6.52, N 11.54. ^{1}H-NMR (CD_3OD + D_2O): 7.77 (s, 1H) H-6; 6.28 (s, 1H) H-1'; 4.45 (d, 1H, $J_{3,4}$ = 3.3 Hz) H-3'; 3.99 (m, 1H, $J_{4',5'}$ = 3.5 Hz, $J_{4',5''}$ = 2.8 Hz) H-4'; 3.80 (m, 2H) H-5' and H-5''; 1.92 (s, 3H) 5 CH_3; ^{13}C-NMR (CD_3OD + D_2O): 88.5 (d,

J_{CH} = 149.0 Hz) C-4'; 86.4 (d, J_{CH} = 169.7 Hz) C-1; 72.0 (d, J_{CH} = 151.5 Hz) C-3'; 62.7 (t, J_{CH} = 142.9 Hz) C-5; 12.8 (q, J_{CH} = 146.5 Hz) 5-CH_3. MS (FAB$^-$): calc. for $(M-H)^-$ 243.0950, found 243.0935.

4-Amino-1-(2'-deoxy-2',2''-dideuterio-β-D-erythro-pentofuranosyl)-1H-pyrimidine-2-one (7e).

Cytosine (165 mg, 1.5 mmol) was condensed with compound 17 (400 mg, 1.03 mmol) following the procedure described for 18, to give a mixture of 19 and its α-isomer (489 mg, 96%). This mixture was treated with methanolic ammonia (20 ml) for 24 h at room temeprature. After concentration, the residue was dissolved in water and extracted with chloroform (3 x 15 ml). The aqueous phase was concentrated to a small volume and was applied to a column of Dowex 1-X2 (OH^-) which was eluated with water. First, the α-isomer was collected out and then came the β-isomer. The later fractions were combined and evaporated to give compound 7e. Yield 95 mg (40%). mp. 203-4°C (methanol); UV (water): λ_{max} = 270 nm (ε = 10.600). $C_9H_{11}D_2N_3O_4$ requires: C 47.16, H/D 6.59, N 18 .33; found C 47.23, H/D 6.68, N 18.43. ^{1}H-NMR, ^{13}C-NMR and MS are identical to the product obtained through the first route (described above).

6-chloro-9-(2'-deoxy-2',2''-dideuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl) purine (20).

A mixture of 6-chloropurine (0.23 g, 1.5 mmol) and sodium hydride (80% in oil, 0.468 g, 1.56 mmol) in dry acetonitrile (13 ml) was stirred at room temperature under argon for 30 min. Compound 17 (488 mg, 1.25 mmol) was then added in portions with stirring in 0.5 h and then the stirring was continued for a further period of 20 h. The suspension was filtered and the filtrate was evaporated in vacuo. The residue was purified by silica gel column chromatography. Yield 347 mg (55%). 20 was crystallized form methanol (302 mg, 48%). mp. 106-8°C (methanol). $C_{26}H_{21}D_2ClN_4O_5$ requires: C 61.36, H/D 4.95, N 11.01, Cl 6.97; found C 61.44, H/D 5.07, N 11.09, Cl 7.16. ^{1}H-NMR ($CDCl_3$): 8.67 (s, 1H) H-8; 8.30 (s, 1H) 2H; 7.90 and 7.24 (m, 8H) arom.; 6.58 (s, 1H) H-1'; 5.83 (d, $J_{3',4'}$ = 1.7 Hz, 1H) H-3'; 4.71 (m, 3H) H-4' and H-5', H-5''; 2.49 and 2.37 (s, 3H each) CH_3 on toluoyl; ^{13}C-NMR ($CDCl_3$): 85.3 (d, J_{CH} = 166 Hz) C-1'; 83.2 (d, J_{CH} = 152 - Hz) C-4'; 74.7 (d, J_{CH} = 159 Hz) C-3'; 63.6 (t, J_{CH} = 150 Hz) C-5'. MS (FAB$^-$): calc. for $(M-H)^-$ 507.1404, found 507.1411.

6-Amino-9-(2'-deoxy-2',2''-dideuterio-β-D-erythro-pentofuranosyl)purine (7a).

Compound 20 (260 mg, 0.5 mmol) in methanolic ammonia (5 ml) was heated at 100°C in a sealed tube. After 20 h the solvent was removed and the residue was dissolved in water (10 ml). The solution was extracted with chloroform and ether. Aqueous layer was evaporated in vacuo and the residue was crystallized from water. Yield 102 mg (82%). mp. 203-4°C (water). UV (water): λ_{max} = 259 nm (ε = 13.800). $C_{10}H_{11}D_2N_5O_3$ requires: C 47.42, H/D 5.97, N 27.65; found 47.54, H/D 6.09, N 27.78. ^{1}H-NMR, ^{13}C-NMR and MS are identical to the product obtained through the first route (described above).

2-Amino-6-chloro-9-(2'-deoxy-2',2''-dideuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-purine (21).

2-Amino-6-chloropurine (187 mg, 1.1 mmol) was condensed with compound 17 (410 mg, 1.05 mmol) using the procedure described for the preparation of compound 20. The title compound 21 was crystallized from methanol. Yield 280 mg (51%). mp. 176-8°C (methanol). $C_{26}H_{22}D_2ClN_5O_5$ requires: C 59.60, H/D 5.77, N 13.37 Cl 6.77; found C 59.69, H/D 5.68, N 13.43, Cl 6.84. ^{1}H-NMR ($CDCl_3$): 7.9 (m, 5H) toluoyl and H-8; 7.2 (m, 4H) toluoyl; 6.36 (s, 1H) H-1'; 5.80 (d, $J_{3',4'}$ = 2.2 Hz, 1H) H-3'; 5.35 (broad s, 2H) $-NH_2$; 4.68 (m, 3H) H-4', H-5' and H-5''; 2.43 and 2.39 (6H) CH_3 of toluoyl; ^{13}C-NMR ($CDCl_3$): 84.7 (d, J_{CH} = 163.6 Hz) C-1'; 82.6 (d, J_{CH} = 153.8 Hz)

C-4'; 74.7 (d, J_{CH} = 168.5 Hz) C-3'; 63.6 (t, J_{CH} = 148.9 Hz) C-5'. MS (FAB^-): calc. for $(M-H)^-$ 522.1514, found 522.1484.

2-Acetamido-9-(2'-deoxy-2',2''-dideuterio-3',5'-di-O-acetyl-β-D-erythro-pentofuranosyl)-9H-purine-6(1H)-one (22).

To a solution of compound 21 (260 mg, 0.5 mmol) in diglyme (10 ml) trimethylamine (3 ml) was added. After stirring at room temperature for 2 h tetraethylammonium hydroxide (M, 3 ml) was added and the solution was stirred overnight at 20°C. Volatile matters were removed in vacuo and the residue was evaporated several times to dryness with dry pyridine. The syrup was then dissolved in pyridine (5 ml) and acetic anhydride (1 ml, 11 mmol) and 4,4-dimethylaminopyridine (10 mg) were then added. The mixture was kept at 40-50°C for ca. 30 h. It was then poured into saturated sodium hydrogen carbonate solution (30 ml) which was extracted with chloroform (3 x 20 ml). The organic phase was evaporated and the residue was purified on a silica gel column. Yield 70 mg (35.7%); mp. 190 °C (decomp.). $C_{16}H_{17}D_2O_2N_5O_7$ requires: C 48.61, H/D 5.35, N 17.71; found C 48.54, H/D 5.41, N 17.82. UV (ethanol) λ_{max} = 254 nm (= 16.800). ^{1}H-NMR ($CDCl_3$): 9.56 (broad s, 1H) -NHAc; 7.74 (s, 1H) H-8; 6.20 (s, 1H) H-1'; 5.41 (d, $J_{3',4'}$ = 1.7 Hz, 1H) H-3'; 4.70 (m, 1H) H-4'; 4.40 (m, 2H) H-5', H-5''; 2.32 (s, 3H) N-2- acetyl; 2.13 and 2.10 (2xs, 6H) 3' and 5'-O-acetyl. ^{13}C-NMR ($CDCl_3$): 85.6 (d, J_{CH} = 170.9 Hz) C-1'; 82.4 (d, J_{CH} = 151.4 Hz) C-4'; 74.4 (d, J_{CH} = 157.3 Hz) C-3'; 63.6 (t, J_{CH} = 161.1 Hz) C-5'; MS (FAB^-): calc. for $(M-H)^-$ 394.1332, found 394.1352.

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References:

1. for a review: D.R. Kearns, CRC Critical Reviews in Biochemistry 15, 237 (1984).
2. (a) "Nucleic acid-metal ion interactions", ed. T.G. Spiro, J. Wiley & Sons, 1980
   (b) B. Rosenberg and L. van Camp, Cancer Res. 30 1799 (1970)
   (c) For a review: J. M. Hill, E. Loeb, A. MacLellan, N.O. Hill, A. Khan and J. J. King, Cancer Chemother. Rep. 59, 647 (1975)
   (d) V. Swaminathan and M. Sundaralingam, CRC critical Reviews in Biochemistry 10, 245 (1979).
3. for reviews: (a) "Structure & Motion: Membranes, Nucleic acids & Proteins", eds. E. Clementi, G. Corongiu, M.H. Sarma & R.H. Sarma, Adenine Press, 1984, New York. (b) "Structure & Dynamics: Nucleic acids & Proteins", eds. E. Clementi & R.H. Sarma, Adenine Press, 1983, New York.
4. G.A. Morris, Magn. Reson. Chem. 24, 371 (1986)
5. C. Altona, Recl. Trav. Chim. Pays-Bas., 101, 413 (1982)
6. A. Pullman & B. Pullmann, Quarterly Rev. of Biophysics, 14, 289 (1981)
7. D.G. Davis & A. Bax, J. Am. Chem. Soc. 107, 2820 & 7197 (1985)
8. S. Roy, Y. Hiyama, D.A. Torchia & J.S. Cohen, J. Am. Chem. Soc. 108, 1675 (1986)
9. T. Pathak, H. Bazin and J. Chattopadhyaya, Tetrahedron 42, 5427 (1986)
10. F. Hansske, D. Madej and M.J. Robins, Tetrahedron 40, 125 (1984)
11. M. J. Robins, J.S. Wilson and F. Hansske, J. Am. Chem. Soc. 105, 4059 (1983).
12. J-C. Wu, H. Bazin and J. Chattopadhyaya, (unpublished observation).
13. O. Dahlman, P.J. Garegg, H. Mayer and S. Scramek, Acta Chem. Scand. B40, 15 (1986)
14. C.E. Ballou, J. Am. Chem. Soc. 79, 165 (1957).
15. M. Kiso and A. Hasegawa, Carbohyd. Res.52, 95 (1976).
16. N.B. Dyatkina, A.V. Azhayev, Synthesis p. 961 (1984).
17. M. Hoffer, Chem. Ber. 93, 2777 (1960).
18. H. Griengl et al. J. Med. Chem. 28, 1769 (1985).
19. Z. Kazimierczuk, H.B. Cottam, G.R. Revankar and R.K. Robins, J. Am. Chem. Soc. 106, 6379 (1984).
20. B.A. Otter & J.J. Fox, P. 285 in "Synthetic Proc. in Nucleic Acid Chem.", eds. W.W. Zorbach & R.S. Tipson, Wiley Interscience, 1968, New York