Tetrahedron Vol. 42, No. 19, pp. 5427 to 5441, 1986
Printed in Great Britain.

0040-4020/86 $5.00 + .00
Pergamon Journals Ltd.

SYNTHESIS OF 2'-DEOXY-2'(S)-DEUTERIO AND 2'-DEOXY-2'(R)-DEUTERIO-β-D-NUCLEOSIDES .

T. PATHAK, H. BAZIN and J. CHATTOPADHYAYA*

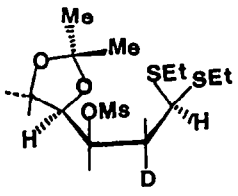
Department of Bioorganic Chemistry, Biomedical Center, Box 581, Uppsala University, S-751 23 Uppsala, Sweden.

(*Received in UK* 28 *July* 1986)

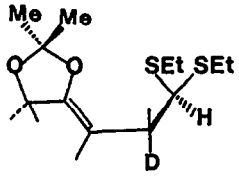
Abstract: Lithium aluminium deuteride (LAD) promoted ring-opening reactions of appropriately protected methyl 2,3-anhydro-α-D-ribofuranoside 2 and methyl 2,3-anhydro-β-D-lyxofuranoside 10, gave 2-deoxy-2(S)-deuterio-erythro-pentose and 2-deoxy-2(R)-threo-pentose derivatives, 4a and 12a, respectively. Compounds 4a and 12a were subsequently converted to 2-deoxy-2(S)-deuterio- and 2-deoxy-2(R)-deuterio-erythro-pentofuranosyl chlorides, 8 and 17 respectively. Thymine, Cytosine, 6-chloropurine and 2-amino-6-chloropurine were then glycosylated with these α-chlorosugars, 8 and 17, to give the corresponding stereospecifically labelled 2-deoxy-2(S)-deuterio- and 2-deoxy-2(R)-deuterio-β-D-nucleosides.

Nuclear Magnetic resonance (NMR) spectroscopy has proved to be a powerful tool for yielding a wealth of information regarding the conformation and dynamics of single and double stranded DNA[1]. Most attention on conformational states of DNA and their interconversions have been focussed on motions of phosphate backbone and the bases using mainly two-dimensional (2D) proton correlated (COSY) and nuclear Overhauser effect (NOESY) data sets. Key steps in assigning and interpreting the complex sugar resonances that arise even from a small oligonucleotide (8 - 12 bases) are to establish connectivities between H-1' to H-2' and H-2'', H-2' to H-3' and H-3' to H-4' by COSY but, before this, it is desirable to establish nOes 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. Despite all these exercises, information on the mobility of sugars is not easily available because of lack of adequate resolution of the sugar proton resonances and the difficulty of labelling sugars regio and stereospecifically with deuterium. An examination of 1D or 2D ^{1}H-NMR spectra of an oligonucleotide immediately reveals that it is the H-2' and H-2'' region of the spectra (5 spin-spin couplings) which contains important information regarding the sugar conformation and is least resolved and is most complex to analyze. We herein report a general approach to the synthesis of 2'-deoxy-2'(S)-deuterio- and 2'(R)-deuterio-nucleosides which provide a means for incremental analysis of DNA molecules by ^{1}H-NMR and observe the sugar mobility by ^{2}H-NMR[2]. It was clear that one of the prerequisite step for the synthesis of such target molecules is to develop convenient synthetic procedures for stereospecific deuterium labelling of the C-2 of the 2-deoxyribose and then condense it with the appropriate aglycone to give the target compounds. This was considered a logical step in view of the unsuccessful reports[3,4] of preparation of the target compounds from 2'-deoxy-2'-chloro nucleosides[3] or a 2'-O-phenoxythiocarbonyl[4] derivative of a ribonucleoside giving a mixture of epimers of 2'-deuterio-2'-deoxynucleosides which is not useful to build any specifically deuterated DNA molecules for NMR work.

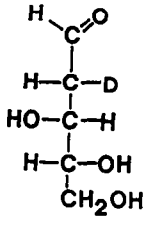
Of the three reports[5-7] for the stereospecific incorporation of deuterium at the C-2 position of a deoxyribose, it is the work by Gray and Wong[7] which is convenient for making the 2-deoxy-2(S)-deuterio-dithioacetal derivative in a large scale. However, the conversion to this 2-deuterio-dithioacetal to the corresponding methylacetal derivative involved highly toxic mercuric chloride and mercuric oxide and did not work satisfactorily in our hands with reproducible yields. To our knowledge, there is only one report[5] of the preparation of 2-deoxy-2(R)-deuterio-D-erythro-pentose which was used for the synthesis of 2'-deoxy-2'(R)-deuteriocytidine. This route involves an assymmetric reduction of a methyl α- D-erythro-hex-2-enopyranoside by lithium aluminium deuteride and then a Lemieux-Johnson oxidation. However, the synthetic steps involved in this strategy were not satisfactory for large preparative runs for 2'-deoxy-2'(R)-deuterio- nucleosides needed for DNA synthesis. An approach could be the synthesis of 2-deoxy-2(R)-deuterio-D-threo-pentose by the reduction of ketene dithioacetal[7], followed by an inversion at C-3 either before or after the ring closure. Our preliminary studies showed a considerable formation of elimination product B with the open chain compound A, while an attempted cyclization of C gave all four isomers and an unfavourable ratio of furanoside over the pyranoside. At this stage, we turned our attention to the work of Taniguchi et. al.[8] who have elegantly shown a preferential S_N2 hydride ion attack at the C-2 of methyl 2,3-anhydro-5-O-benzyl-α-ribofuranoside (3) and methyl 2,3- anhydro-5-O-benzyl-β-D-lyxofuranoside (11) giving the 2-deoxy- erythro-pentose and the 2-deoxy-threo-pentose which were isolated as their acetates in 64 and 51% yields, respectively. We reasoned that the increase of the bulk of the protective group of the C-5 hydroxy function may enhance the C-2 regioselectivity of the hydride-promoted S_N2 opening of the epoxide. It was found actually to be the case that



A



B

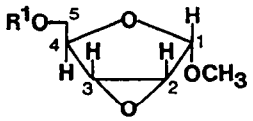


C

the selectivity of the hydride ion attack at the C-2 vs. C-3 could be improved to ca. 9:1, respectively, in 2 and 10, when the C-5 was derivatized with the 4-monomethoxytrityl (MMTr) group.

Synthesis of 2-deoxy-2(S)-deuterio-α-D-erythro-pentofuranosyl chloride (8)

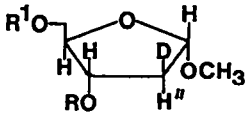
The key starting material, methyl 2,3-anhydro-α-D-ribofuranoside (1) was easily accessible in a large scale[9]. Treatment of 1, in dry pyridine solution, with MMTr-Cl gave 2 in 75% crystalline yield. Compound 2, upon treatment with lithium aluminium deuteride (LAD, 98% atom D) (2 equiv.) gave an inseparable mixture of 4a and its C-3 isomer 4b in 9:1 ratio (NMR) in 95% yield. Attempts to remove the MMTr group from the mixture of isomers (4a + 4b) by 80% aqueous acetic acid or by trifluoroacetic acid caused a considerable breakdown of the sugar. The mixture of isomers (4a + 4b) were, therefore converted to their toluoyl derivatives (5a + 5b) in the usual way, in a quantitative yield, which were then treated with 80% aqueous acetic acid to give the mixture of isomers (6a + 6b) in 81% yield after a column chromatographic purification step. Isomers 6a + 6b were then converted to their bis-toluate derivatives (7a + 7b) in 89% yield. At this stage, the desired pure 2-deoxy-2(S)-deuterio-pentose derivative (7a) was crystallized out from methanol to homogeneity (NMR) in 75% yield from the mixture of isomers. The crystalline isomer 7a was then converted to its α-chlorosugar 8 following a literature procedure[10].



1: R^1 = H

2: R^1 = MMTr

3: R^1 = Bn

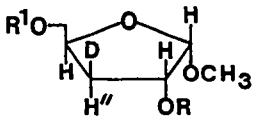


4a: R = H, R^1 = MMTr

5a: R = Tol, R^1 = MMTr

6a: R = Tol, R^1 = H

7a: R = R^1 = Tol

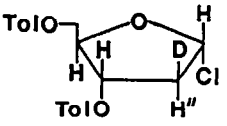


4b: R = H, R^1 = MMTr

5b: R = Tol, R^1 = MMTr

6b: R = Tol, R^1 = H

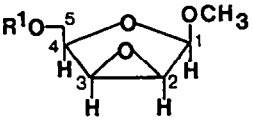
7b: R = R^1 = Tol



8

Synthesis of 2-deoxy-2(R)deuterio-α-D-erythro-pentofuranosyl chloride (17)

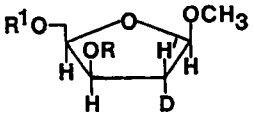
The key starting material, methyl 2,3-anhydro-β-D-lyxofuranoside 9 was prepared in a large scale (20 g) using a literature procedure[9] which was converted to its C-5 monomethoxytrityl derivative 10 in 84% yield. Compound 10, upon LAD treatment (2 equiv.) gave a mixture of 2- and 3-deoxy-threo-pentose derivatives 12a and 12b in ca. 9:1 ratio, respectively. Upon a standard flash chromatographic purification of the latter mixture on silica gel, the desired 2-deoxy-2(R)-deuterio-threo-pentose derivative 12a could be isolated in a pure form in 82% yield. Compound 12a was then converted to its C-3 mesylate 13 in an almost quantitative yield and then the C-5 MMTr



9: R^1 = H

10: R^1 = MMTr

11: R^1 = Bn

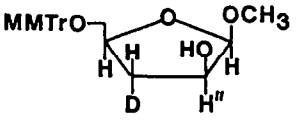


12a: R = H, R^1 = MMTr

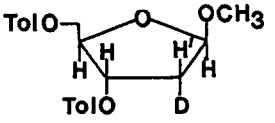
13: R = Ms, R^1 = MMTr

14: R = Ms, R^1 = H

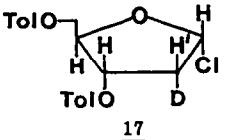
15: R = Ms, R^1 = Tol



12b

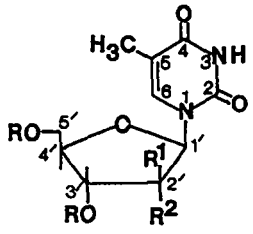


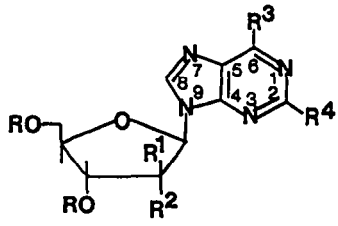
16



17

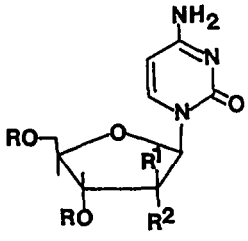
group was cleanly removed with 80% aqueous acetic acid, without any anomerisation or conversion to the corresponding aldose, to give 14 in 76% yield. The primary hydroxyl function of 14 was subsequently protected with the toluoyl group in a standard way to give 15 in 92% yield. An inversion of the C-3 configuration of 15, by sodium-p-toluate induced $S_{N}2$ reaction in 6% aqueous DMF gave the crystalline erythro-bis-toluate derivative 16 in 71% yield which gave the α-chlorosugar 17 in 74% yield by the action of HCl in acetic acid according to a reported procedure[10].

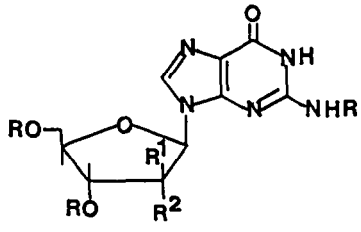




18a: R = Tol, R^1 = D, R^2 = H"
18b: R = Tol, R^1 = H', R^2 = D
19a: R = H, R^1 = D, R^2 = H"
19b: R = H, R^1 = H', R^2 = D

22a: R = Tol, R^1 = D, R^2 = H", R^3 = Cl, R^4 = H
22b: R = Tol, R^1 = H', R^2 = D, R^3 = Cl, R^4 = H
23a: R = H, R^1 = D, R^2 = H", R^3 = NH_2, R^4 = H
23b: R = H, R^1 = H', R^2 = D, R^3 = NH_2, R^4 = H
24a: R = Tol, R^1 = D, R^2 = H", R^3 = Cl, R^4 = NH_2
24b: R = Tol, R^1 = H', R^2 = D, R^3 = Cl, R^4 = NH_2





20a: R = Tol, R^1 = D, R^2 = H"
20b: R = Tol, R^{11}= H', R^2 = D
21a: R = H, R^1 = D, R^2 = H"
21b: R = H, R^1 = H', R^2 = D

25a: R = H, R^1 = D, R^2 = H"
25b: R = H, R^1 = H', R^2 = D
26a: R = Ac, R^1 = D, R^2 = H"
26b: R = Ac, R^1 = H', R^2 = D

Synthesis of 2'-deoxy-2'(S)deuterio- and 2'-deoxy-2'(R)deuterio-nucleosides.

The 2-deoxy-2(S)-deuterio- -chlorosugar 8 was subsequently used for condensation reactions with thymine[11], 6-chloropurine[12] and 2-amino-6-chloropurine to give the corresponding 2'-deoxy-2'(S)deuterio-nucleosides, 18a, 22a and 24a in 59, 42 and 40% yields, respectively. The condensation[11] of 8 and cytosine gave a mixture of 20a and the corresponding α nucleoside which could not be separated in the protected form. All condensation reactions were carried out using literature procedures[11,12] except for the condensation of 8 with 2-amino-6-chloropurine. This was carried out through the preparation of the sodium salt of 2-amino-6-chloropurine using sodium hydride in dry acetonitrile, as described for the preparation of 22a. Such a condition gave 24a, free of the corresponding α isomer, while other reported procedures[13] gave a complex mixture of α and β isomers. Compounds 18a, 22a were then converted to the free nucleosides, 19a and 23a in 96 and 71% yields respectively. The mixture of compounds 20a + α isomer were deprotected using literature procedure[11] and separated on a Dowex-OH^- column to obtain pure 21a in 47% yield. Compound 24a was also converted to 25a using a literature procedure[13], however it was isolated as its triacetate 26a.

The 2-deoxy-2(R)-deuterio-α-chlorosugar 17 was then condensed, in a similar manner[11,12], with thymine, 6-chloropurine and 2-amino-6-chloropurine to give the corresponding crystalline 2'-deoxy-2'(R)-deuterio-nucleosides, 18b, 22b and 24b in 89, 56 and 51% yields, respectively. Compounds 18b and 22b have been subsequently converted to free nucleosides, 19b and 23b, in 96 and 77 % yields, respectively. Compound 24b was also converted to free nucleoside 25b, however it was isolated as its triacetate 26b. As before, condensation of the chlorosugar 17 with cytosine gave a mixture of 20b + its α isomer which were not separable and, therefore, they were deprotected and separated in the usual way to give pure 2'-deoxy-2'(R)deuteriocytidine 21b in 63% yield.

The ^{1}H-NMR spectra of the 2'-deoxy-2'(S)-deuterio- and 2'-deoxy-2'(R)- deuterio-nucleosides, Panel B and Panel C, respectively, are shown in Figures 1, 2, 3 and 4 respectively while the corresponding non-deuterated (natural) nucleosides are shown in the panel A. A comparison of these spectra in panels A, B and C in figures 1 to 4 clearly illustrate the specific labellings that are achieved in the present work. The spectra in Figures 1 to 4 also establish that an absence of the geminal H-2' and H-2" spin-spin coupling and other vicinal couplings, in the 2'-deoxy-2'(S)-deuterio- and 2'-deoxy-2'(R)-deuterio-nucleosides, do indeed simplify the ^{1}H-NMR spectra considerably. The application of this work lies in the selective incorporation of 2'-deoxy-2'(S)deuterio- and 2'-deoxy-2'(R)- deuterio-nucleosides in oligo-DNA which would assist in the assignment and analysis of otherwise least resolved and most complex resonances that arise from the sugar part of the DNA molecule.

Work is now in progress in this laboratory to synthesize DNA segments with these deuterium labelled nucleosides in order to gain information on the sugar mobility by ^{1}H- and ^{2}H-NMR spectroscopy.

Experimental

Melting points were uncorrected. ^{1}H-NMR spectra at 90 MHz and 270 MHz were recorded with Jeol FX 90Q and Jeol 270 MHz FT spectrometer. ^{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. Thin-layer chromatography (t.l.c.) was performed on Merck precoated 60 F_{254} plates. Merck Kieselgel G was used for short column chromatography.

Methyl-2,3-anhydro-5-O-(4-methoxytriphenylmethyl)-α-D-ribofuranoside (2). Methyl 2,3-anhydro-α-D-ribofuranoside (1) (4 g, 27.4 mmol) was dissolved in a small volume of pyridine (ca. 5 ml) and volatile matters were removed in vacuo. This operation was repeated three times. Dry pyridine (100 ml) was added to it followed by 4-methoxytriphenylmethyl (MMTr) chloride (12.1 g, 41.1 mmol). The mixture was stirred overnight at 20 °C, saturated sodium hydrogen carbonate solution (15 ml) was added and stirred for 30 min. This mixture was partitioned between saturated sodium hydrogen carbonate solution and dichloromethane (4 x 60 ml). Organic layers were pooled and evaporated and co-evaporated a few times with toluene in vacuo. The residue was then dissolved in ethanol, and evaporated, whereby the title compound crystallized out. yield 8.6 g (75%) mp. 131-2 °C. ^{1}H NMR ($CDCl_3$): 7.24-6.78 (m, 14H) arom.; 5.34 (s, 1H) H-1; 4.37 (t,

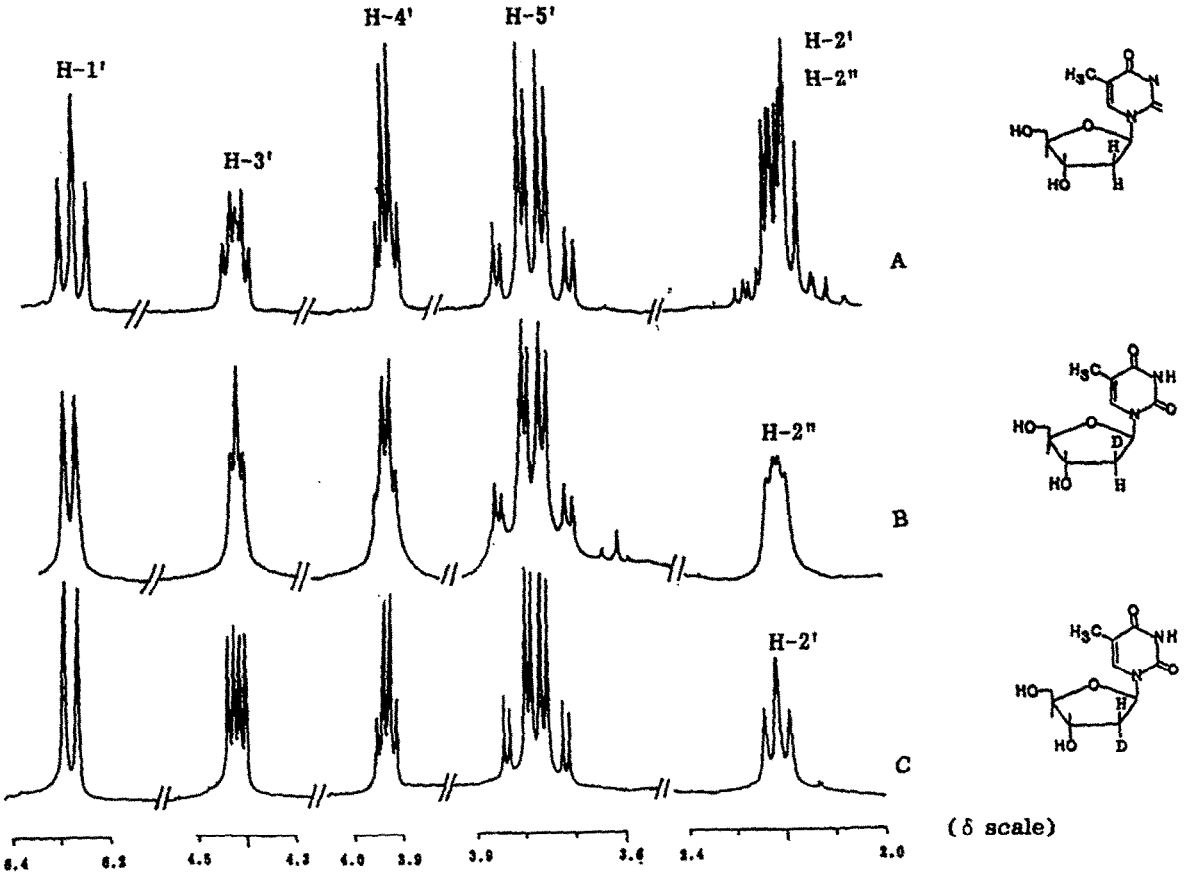


FIGURE 1

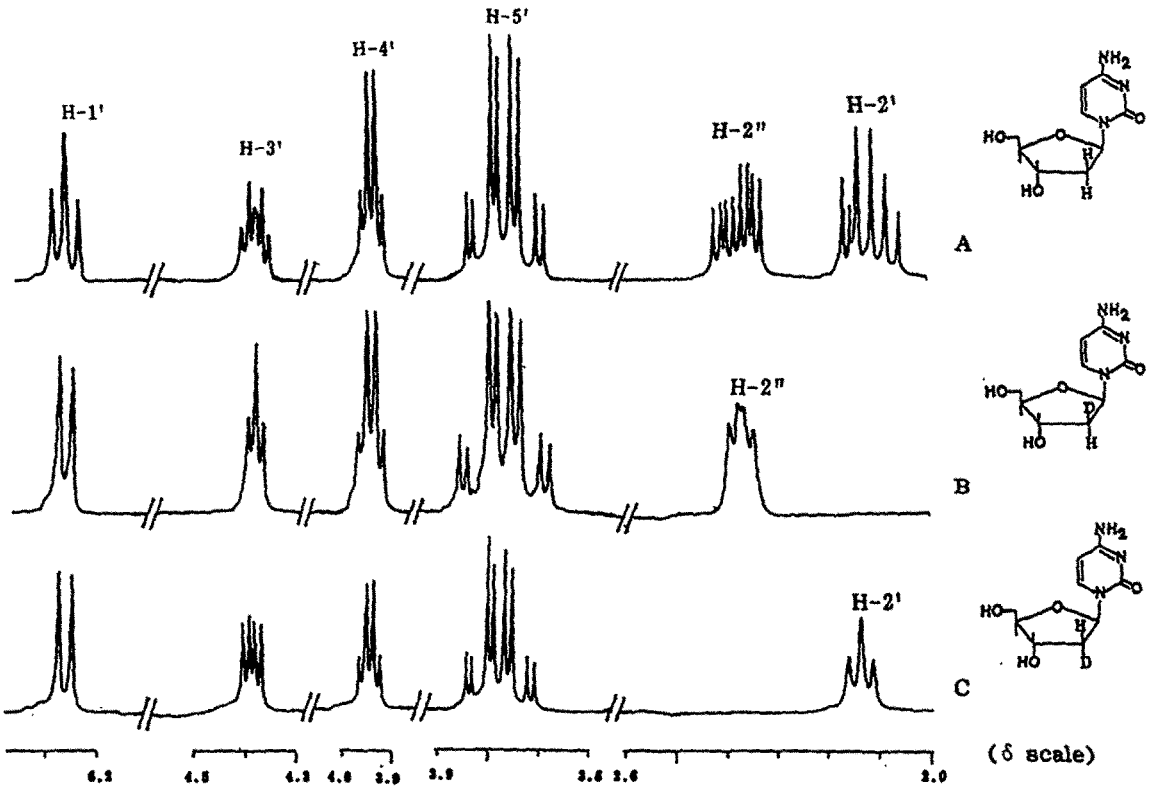


FIGURE 2

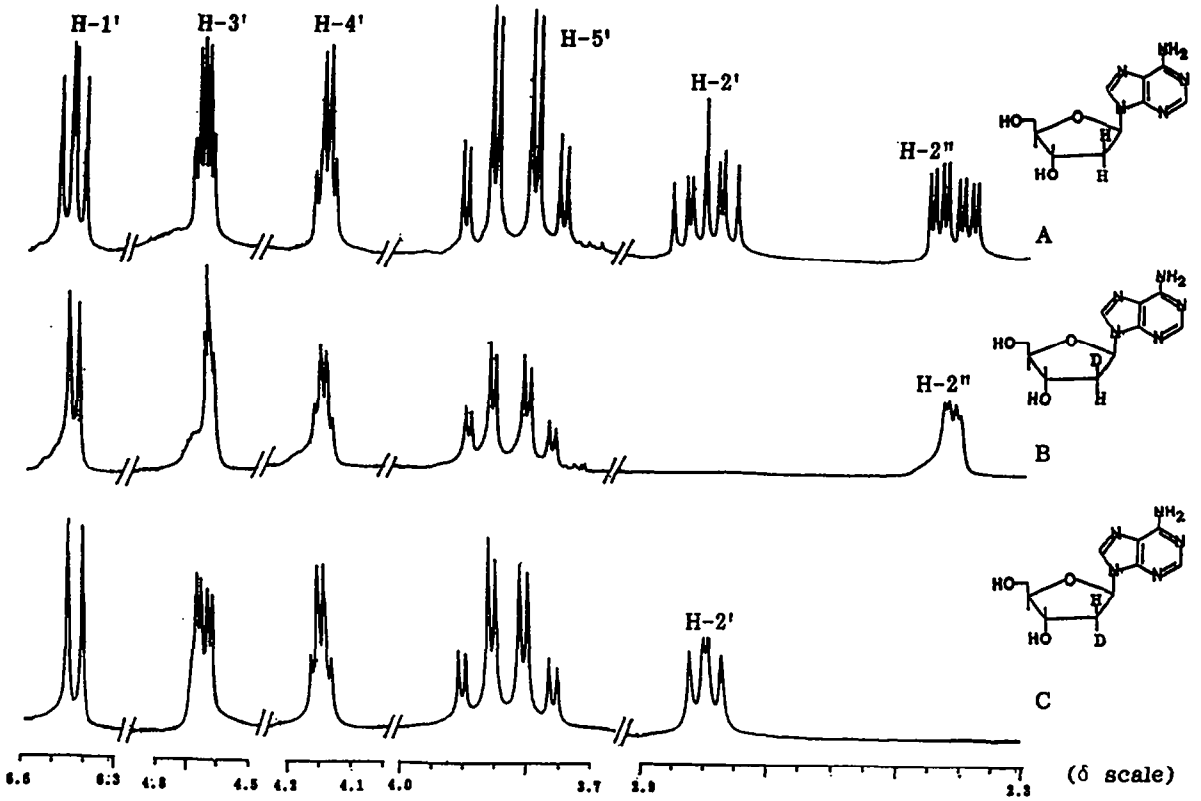


FIGURE 3

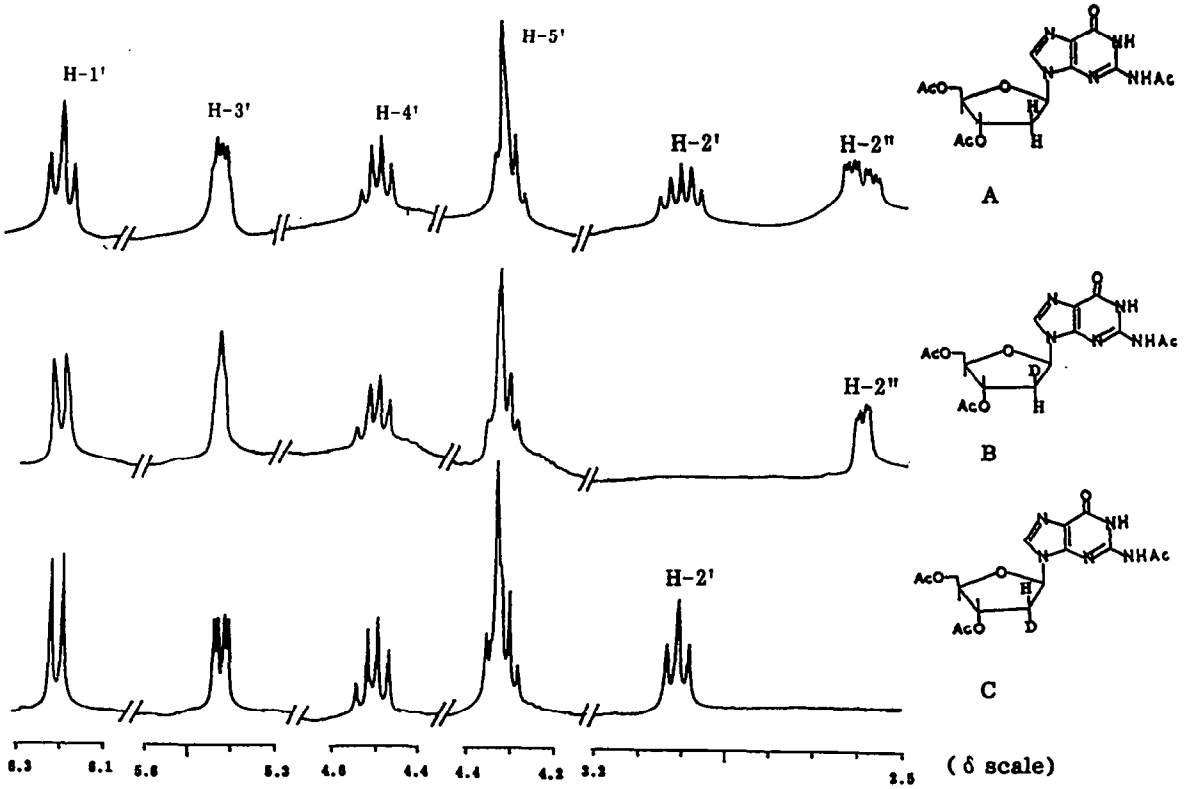


FIGURE 4

$J_{4,5}$ = 3.6 Hz) H-4; 3.85 (d, $J_{2,3}$ = 2.9 Hz, 1H) H-3; 3.79 (s, 3H) methoxy; 3.65 (d, $J_{2,3}$ = 2.9 Hz, 1H) H-2; 3.55 (s, 3H) methoxy of C-1; 3.24 (m, 2H) H-5. ^{13}C NMR ($CDCl_3$): 102.8 (d, J_{CH} = 161.7 Hz) C-1; 77.5 (d, J_{CH} = 149.4 Hz) C-4; 64.1 (t, J_{CH} = 143.1 Hz) C-5; 56.8 (q, J_{CH} = 142.0 Hz) methoxy from C-1; 56.1 (d, J_{CH} = 183.1 Hz) C-2, C-3; 55.1 (q, J_{CH} = 143.1 Hz) methoxy of MMTr. Calc. for $C_{26}H_{26}O_5$: C, 74.64; H, 6.22. Found: C, 74.57; H, 6.12.

Methyl 2-Deoxy-2(S)-deuterio-5-O-(4-methoxytriphenylmethyl)-α-D-ribofuranoside (4a) and its C-3 - isomer (4b). To a suspension of lithium aluminium deuteride (98% atom D, 0.4 g, 10 mmol) in dry tetrahydrofuran (50 ml), cooled at 0°, was added dropwise a solution of 2 (2.1 g, 5 mmol) in the same solvent (50 ml). The mixture was stirred for 6 h, then quenched with a 4% aqueous sodium hydroxide solution (1.6 ml) and stirred for 30 min. The mixture was filtered and the filtrate was extracted with dichloromethane (3 x 25 ml). The organic layer was washed with water, dried ($MgSO_4$), filtered and the filtrate was evaporated to dryness. The oily residue was purified by silica gel column chromatography to give the title compound 4a, contaminated with the other isomer 4b, in the 9:1 ratio (NMR). Yield 1.9 g (95%). ^{1}H NMR ($CDCl_3$): 7.28 and 6.87-6.77 (m, 14H) arom.; 5.14 (s, 1H) H-1 of 4a; 4.92 (d, $J_{1,2}$ = 4.1 Hz) H-1 of 4b; 4.18 (m, 2H) H-3 and H-4; 3.79 (s, 3H) methoxy of MMTr; 3.38 (s, 3H) methoxy of C-1; 3.14 (m, 2H) H-5 and 5'; 1.99 (s, 1H) H-2". ^{13}C NMR ($CDCl_3$): 105.4 (d, J_{CH} = 178.6 Hz) C-1 of 4a; 102.8, C-1 of 4b; 86.5 (d, J_{CH} = 147.1 Hz) C-4, 73.1 (d, J_{CH} = 152.7 Hz) C-3; 64.1 (t, J_{CH} = 141.5 Hz) C-5; 54.9 (q, J_{CH} = 143.8) methoxy of MMTr; 54.7 (q, J_{CH} = 146.0 Hz) methoxy of C-1; 40.7 (t, J_{CD} = 19.6 Hz) C-2.

Methyl 2-Deoxy-2(S)-deuterio-3-O-toluoyl-α-D-ribofuranoside (6a) and the C-3 isomer (6b). The mixture of 4a and 4b (2.5 g, 6 mmol) was dried by coevaporation with pyridine. The syrup was dissolved in dry pyridine (60 ml) and toluoyl chloride (1.2 ml, 9 mmol) was added. The mixture was stirred at 20 °C for 1 h and then heated at 40 °C for 2 h. It was then cooled to 20 °C. saturated sodium hydrogen carbonate solution (ca. 5 ml) was added and the mixture was partitioned between chloroform (3 x 25 ml) and saturated sodium hydrogen carbonate (50 ml) solution. Organic phase was dried in vacuo and pyridine was removed by coevaporation with toluene (4 x 50 ml) to give 5a + 5b quantitatively which was dissolved in a small volume of tetrahydrofuran and 80% aq. acetic acid solution (60 ml) was added. The solution was stirred for 4 h at 20 °C. Solvent was removed in vacuo and the residual acetic acid was removed by coevaporations with toluene. The residue was purified by silica gel column chromatography to give an oil. Yield: 1.36 g (81%). ^{1}H NMR ($CDCl_3$): 7.98-7.89 and 7.28-7.18 (m, 4H) arom.; 5.31 (t, 1H) H-3; 5.18 (d, $J_{1,2}$ = 2.7 Hz) H-1 of 6b; 5.15 (s, 1H) H-1 of 6a; 4.25 (q, 1H) H-4; 3.87 (m, 2H) H-5, 5'; 3.41 (s, 3H) methoxy; 2.41 (s, 3H) methyl; 2.18 (s, 1H) H-2''. ^{13}C NMR ($CDCl_3$): 104.9 (d, J_{CH} = 170.7 Hz) C-1 of 6a; 101.9 (d, J_{CH} = 174.1 Hz) C-1 of 6b; 83.7 (d, J_{CH} = 150.5 Hz) C-4; 74.5 (d, J_{CH} = 164.1 Hz) C-3; 62.5 (t, J_{CH} = 141.5 Hz) C-5; 54.9 (q, J_{CH} = 143.7 Hz) methoxy; 39.0 (t, J_{CD} = 19.6 Hz) C-2.

Methyl-2-deoxy-2(S)-deuterio-3,5-di-O-toluoyl-α-D-ribofuranoside (7a). The mixture of 6a + 6b (1.3 g, 4.8 mmol) was dried by coevaporation with pyridine and was dissolved in dry pyridine (25 ml). Toluoyl chloride (1.11 g, 7.2 mmol) was added to it and the mixture was stirred overnight at 20 °C. It was worked up as described for the preparation of 6a + 6b. The oil was purified by silica gel column chromatography and the title compound was crystallized out from methanol free of the minor isomer. Total crude yield: 1.6 g (89%); Yield of the pure title compound 1.2 g (75%). mp. 81 - 2 °C. ^{1}H NMR ($CDCl_3$): 7.93 and 7.22 (m, 8H) arom.; 5.41 (dd, $J_{3,4}$ = 2.4 Hz, 1H) H-3; 5.19 (s, 1H) H-1; 4.63 (m, 1H), H-4; 4.54 (m, 2H) H-5; 3.43 (s, 3H) methoxy; 2.4 (s, 6H) arom. methyl; 2.18 (s, 1H) H-2". ^{13}C NMR ($CDCl_3$): 104.9 (d, J_{CH} = 171.8 Hz) C-1; 81.0 (d, J_{CH} = 150.5 Hz) C-4; 74.6 (d, J_{CH} = 153.9 Hz) C-3; 64.3 (t, J_{CH} = 148.8 Hz) C-5; 54.9 (q, J_{CH} = 138.1 Hz) methoxy; 38.9 (t, J_{CD} = 20.1 Hz) C-2. Calc. for $C_{22}H_{23}DO_6$: C, 68.57; H/D, 6.49. Found: C, 68.38; H/D, 6.23. MS (FAB$^-$): calc. for $(M-H)^-$ 384.1557, found 384.1600.

2-Deoxy-2(S)-deuterio-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride (8). Compound 7a (1.9 g, 5 mmol) was converted to the title compound following the literature procedure[10]. Yield: 1.46 g (77%). mp. 116 - 8 °C. ^{1}H NMR ($CDCl_3$): 7.9 (m, 4H) and 7.2 (m, 4H) arom.; 6.46 (s, 1H) H-1; 5.55 (d, 1H) H-3; 4.85 (q, 1H) H-4; 4.62 (m, 2H) H-5; 2.72 (s, 1H) H-2"; 2.42 and 2.41 (2 x s, 6H) arom. methoxy. ^{13}C NMR ($CDCl_3$): 95.3 (d, J_{CH} = 189.7 Hz) C-1; 84.7 (d, J_{CH} = 153.9 Hz) C-4; 73.5 (d, J_{CH} = 156.7 Hz) C-3; 63.5 (t, J_{CH} = 148.8 Hz) C-5; 44.3 (t, J_{CD} = 18.8 Hz) C-2. Calc. for $C_{21}H_{20}DClO_5$: C, 64.69; H/D, 5.64; Cl, 9.11. Found: C, 64.47; H/D, 5.4; Cl, 9.05.

1-(2'-Deoxy-2'(S)-deuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pento-furanosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione (18a). A suspension of thymine (0.12 g, 0.97 mmol) in a mixture of hexamethyldisilazane (2 ml) and trimethylchlorosilane (0.2 ml) was refluxed until it turned into a clear solution. Volatile matters were removed in vacuo and co-evaporated with dry xylene. The syrup was dissolved in chloroform (5 ml) and compound 8 (0.34 g, 0.88 mmol) was added. The mixture was stirred for 24 h at 20 °C. The resultant solution was diluted with chloroform and extracted with water (3 x 10 ml). Organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified on a silica gel column to give a mixture of α and β isomers from which compound (18a) was crystallised out using 95% ethanol. Yield: 250 mg (59.5%); mp. 196-7 °C. ^{1}H NMR ($CDCl_3$): 7.95 and 7.26 (m, 9H) arom. and H-6; 6.49 (d, $J_{1',2"}$ = 5.6 Hz) H-1'; 5.64 (bs, 1H) H-3'; 4.72 (m, 2H) H-5' and 5''; 4.55 (m, 1H) H-4'; 2.69 (dd, $J_{1',2"}$ = 5.5 Hz and $J_{2",3'}$ = 1.3 Hz, 1H) H-2''; 2.42 (s, 6H) CH_3- toluoyl; 1.61 (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} = 150.5 Hz) C-4'; 74.9 (d, J_{CH} = 160 Hz) C-3'; 64.3 (t, J_{CH} = 150.5 Hz) C-5'; 37.8 (t, J_{CD} = 20 Hz) C-2'. Calc. for $C_{26}H_{25}DN_2O_7$: C, 65.13; H/D, 5.64; N, 5.84. Found: C, 64.95; H/D, 5.3; N, 5.73. MS (FAB$^-$): calc. for $(M-H)^-$ 478.1725, found 478.1698.

1-(2'-Deoxy-2'(S)-deuterio-β-D-erythro-pentofuranosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione (19a). Compound 18a (200 mg, 0.42 mmol) was dissolved in methanolic ammonia (25 ml) and the solution was stirred overnight. Volatile matters were removed in vacuo. The residue was dissolved in water and extracted with ether. Aqueous phase was concentrated to dryness to give 19a. Yield: 97 mg (96%) mp. 182 - 4 °C (methanol). UV (ethanol): λ_{max} = 267 nm (ε = 8.800) pH 7. ^{1}H NMR (CD_3OD): 7.8 (d, $J_{5\text{-}CH_3,6}$ = 1.3 Hz, 1H) H-6; 6.28 (d, $J_{1',2"}$ = 6.0 Hz, 1H) H-1'; 4.39 (t, $J_{2",3'}$ = 3.3 Hz, 1H) H-3'; 3.9 (m, 1H) H-4'; 3.76 (m, 2H) H-5',5''; 2.2 (dd, $J_{1',2"}$ = 5.9 Hz and $J_{2",3'}$ = 3.3 Hz) H-2"; 1.88 (d, $J_{5\text{-}CH_3,6}$ = 1.1 Hz, 3H) methyl. ^{13}C NMR (CD_3OD): 88.7 (d, J_{CH} = 148.3 Hz) C-4'; 86.1 (d, J_{CH} = 165.1 Hz) C-1'; 72.1 (d, J_{CH} = 148.3 Hz) C-3'; 62.7 (t, J_{CH} = 139.3 Hz) C-5'; 40.8 (t, J_{CD} = 20.2 Hz) C-2'. Calc. for $C_{10}H_{13}DN_2O_5$: C, 49.38; H/D, 6.17; N, 11.52. Found: C, 49.5; H/D, 5.91; N, 11.3. MS (EI$^+$): calc. for M^+ 243.0966, found 243.0949.

4-Amino-1-(2'-deoxy-2'(S)-deuterio-β-D-erythro-pentofuranosyl)-1H-pyrimidine-2-one (21a). Cytosine (177 mg, 1.6 mmol) was condensed with compound 8 (630 mg, 1.6 mmol) following the procedure as described for 18a to afford a mixture of 20a and α isomer in 96% yield. This mixture (610 mg, 1.3 mmol) was treated with 1 N sodium methoxide in methanol and stirred at 20 °C until the reaction was complete. After neutralization with 1 N acetic acid, the solvents were removed in vacuo and co-evaporated with water. The residue was dissolved in water (10 ml) and extracted with chloroform (3 x 15 ml) and ether (3 x 20 ml). The aqueous part was evaporated to dryness. This residue was dissolved in small volume of water and was applied to a column of Dowex 1-X2 ($^-$OH) (100 g) packed in the same solvent. Water was used to elute the column. Fraction 25-49 were pooled together and evaporated (fraction A_1). Fraction 59-95 were pooled together and evaporated (fraction B_1). Fraction 50-58 were pooled together, evaporated and refractionated in

the same way (fractions A_2 and B_2). Fractions B_1 and B_2 mixed together and evaporated to afford compound 21a. Yield: 140 mg (47%). mp. 204-6 °C (methanol). UV (water): λ_{max} = 271 nm (ε = 8.200) pH 7. ^{1}H NMR (CD_3OD): 7.9 (d, $J_{5,6}$ = 7.5 Hz, 1H) H-6; 6.25 (d, $J_{1',2''}$ = 6.0 Hz, 1H) H-1'; 5.9 (d, $J_{5,6}$ = 7.5 Hz, 1H) H-5; 4.36 (t, $J_{2'',3'}$ = 3.6 Hz, 1H) H-3'; 3.93 (m, 1H) H-4'; 3.75 (m, 2H) H-5''; 2.33 (dd, $J_{1',2''}$ = 6.0 Hz and $J_{2'',3'}$ = 3.8 Hz, 1H) H-2". ^{13}C NMR (CD_3OD): 88.8 (d, J_{CH} = 148.8 Hz) C-4'; 87.5 (d, J_{CH} = 169.6 Hz) C-1'; 71.9 (d, J_{CH} = 147.1 Hz) C-3'; 62.7 (t, J_{CH} = 140.9 Hz) C-5'; 41.7 (t, J_{CD} = 19.1 Hz) C-2'. Calc. for $C_9H_{12}DN_3O_4$: C, 47.37; H/D, 6.13; N, 18.42. Found: C, 47.1; H/D, 6.07; N, 18.62. MS (FAB^-): calc. for $(M-H)^-$ 227.0891, found 227.0886.

6-chloro-9-(2'-deoxy-2'(S)-deuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl) purine (22a). A mixture of 6-chloropurine[14] (0.58 g, 3.78 mmol) and sodium hydride (80% in oil, 0.117 g, 3.93 mmol) in dry acetonitrile (25 ml) was stirred at 20 °C under argon for 30 min. Compound 8 (1.34 g, 3.44 mmol) was added in portions with stirring and the stirring was continued for 24 h. The suspension was filtered and all volatile matters were removed in vacuo to give an oil which was then purified on a silica gel column. Compound, which eluted first was crystallized from 95% ethanol and was shown to be compound 22a. Yield: crude 951 mg (54%); crystallized yield (methanol): 740 mg (42%). mp. 106 - 9 °C. ^{1}H NMR ($CDCl_3$): 8.66 (s, 1H) H-8; 8.31 (s, 1H) H-2; 7.9 - 7.25 (m, 8H) arom.; 6.57 (d, $J_{1',2''}$ = 5.8 Hz, 1H) H-1'; 5.84 (t, $J_{2'',3'}$ = 2.2 Hz and $J_{3',4'}$ = 2.1 Hz, 1H) H-3'; 4.78 (m, 1H) H-4'; 4.67 (m, 2H) H-5',5''; 2.88 (dd, $J_{1',2''}$ = 5.8 Hz and $J_{2'',3'}$ = 2.2 Hz) H-2"; 2.43 (s, 3H), 2.39 (s, 3H) toluoyl-methyl. ^{13}C NMR: 85.2 (d, J_{CH} = 166.9 Hz) C-1'; 83.2 (d, J_{CH} = 153.0 Hz) C-4'; 74.7 (d, J_{CH} = 155.5) C-3'; 63.5 (t, J_{CH} = 150.3 Hz) C-5'; 37.4 (t, J_{CD} = 18.3 Hz) C-2'. Calc. for $C_{26}H_{22}DClN_4O_5$: C, 61.47; H/D, 4.72; N, 11.03. Found: C, 61.3; H/D, 4.52; N, 11.17. MS (EI^+): calc. for M^+ 507.1421, found 507.1401.

6-Amino-9-(2'-deoxy-2'(S)-deuterio-β-D-erythro-pentofuranosyl)purine (23a). Compound 22a (507 mg, 1 mmol) was heated at 100 °C in methanolic ammonia (10 ml). After 15 h solvent was removed and the residue was dissolved in water (15 ml). The solution was extracted first with chloroform and then with ether (3 x 15 ml). Aqueous layer was collected and evaporated in vacuo; the residue was crystallized from water. Yield: 0.18 g (71%). mp. 185 °C. UV (ethanol): λ_{max} 259 nm (ε = 13.800) pH 7. ^{1}H NMR (CD_3OD): 8.31 (s, 1H) H-8; 8.18 (s, 1H) H-2; 6.43 (d, $J_{1',2''}$ = 6 Hz, 1H) H-1'; 4.59 (t, $J_{2'',3'}$ = 2.4 Hz, 1H) H-3'; 4.08 (m, 1H) H-4'; 3.8 (m, 2H) H-5',5''; 2.39 (dd, $J_{1',2''}$ = 6.0 Hz and $J_{2'',3'}$ = 2.4 Hz, 1H) H-2". ^{13}C NMR (CD_3OD + D_2O): 89.6 (d, J_{CH} = 148.3 Hz) C-4'; 86.8 (d, J_{CH} = 164.0 Hz) C-1'; 72.8 (d, J_{CH} = 151.6 Hz) C-3'; 63.4 (t, J_{CH} = 141.0 Hz) C-5'; 41.2 (t, J_{CD} = 19.7 Hz) C-2'. Calc. for $C_{10}H_{12}DN_5O_3$: C, 47.62; H/D, 5.55; N, 27.77. Found: C, 47.51; H/D, 5.1; N, 27.93. MS (EI^+): calc. for M^+ 252.1081, found 252.1051

2-Amino-6-chloro-9-(2'-deoxy-2'(S)-deuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl) purine (24a). 2-Amino-6-chloropurine (0.34 g, 2 mmol) was condensed with compound 8 (0.78 g, 2 mmol) using a procedure described for the preparation of compound 22a. Title compound 24a was crystallized from ethanol. Yield: Crude 540 mg (52%), crystallized 420 mg (40%). mp. 175-8 °C. ^{1}H NMR ($CDCl_3$): 7.89 (m, 5H) H-8 and arom.; 7.26 (m, 4H) arom.; 6.36 (d, $J_{1',2''}$ = 6.1 Hz, 1H) H-1'; 5.8 (t, $J_{2'',3'}$ = 2.2 Hz and $J_{3',4'}$ = 1.9 Hz) H-3'; 5.26 (bs, 2H), NH_2; 4.68 (m, 3H) H-4', 5' and 5''; 2.72 (dd, $J_{1',2''}$ = 6.1 Hz and $J_{2'',3'}$ = 2.1 Hz) H-2"; 2.4 (s, 6H) toluoyl-methyl. ^{13}C NMR ($CDCl_3$): 84.8 (d, J_{CH} = 164.0 Hz) C-1'; 82.7 (d, J_{CH} = 152.8 Hz) C-4'; 75.0 (d, J_{CH} = 146.0 Hz) C-3'; 63.8 (t, J_{CH} = 149.4 Hz) C-5'; 36.5, C-2'. Calc. for $C_{26}H_{23}DClN_5O_5$: C, 59.71; H/D, 4.78; Cl, 6.79; N, 13.39. Found: C, 59.51; H/D, 4.92; Cl, 7.01; N, 13.17. MS (FAB^+): calc. for $(M+H)^+$ 523.1607, found 523.1591.

2-Acetamido-9-(2'-deoxy-2'(S)-deuterio-3'-5'-di-O-acetyl-β-D-erythro-pentofuranosyl)-9H-purine-6(1H)-one (26a). Compound 24a (400 mg, 0.76 mmol) was dissolved in methanol (6 ml). Sodium methoxide (0.164 g, 3 mmol) and 2-mercaptoethanol (0.24 ml) were added to the mixture. After adding two drops of water to it, the mixture was heated under reflux for 1.5 h. Another portion of sodium methoxide (0.11 g, 2 mmol) was added and heating under reflux was continued for further 1 h. All volatile matters were removed in vacuo. The solid residue was dissolved in water (15 ml). The solution was neutralised with 80% acetic acid. All volatile matters were removed in vacuo and co-evaoporated with pyridine (5 x 5 ml). The residue was taken up in DMF (4 ml). Pyridine (4 ml), acetic anhydride (1 ml, 11 mmol) and 4-Dimethylaminopyridine (10 mg) were added and the mixture was stirred ca. 30 h at 40-50 °C. It was poured in saturated sodium hydrogen carbonate solution (20 ml) which was extracted with dichloromethane (3 x 10 ml). The organic phase was evaporated and the residue was purified on a silica gel column. Yield: 150 mg (50%). mp. 200 °C (methanol). UV (EtOH): λ_{max} = 256 (ε = 14.000) pH 7. ^{1}H NMR ($CDCl_3$ + CD_3OD): 7.78 (s, 1H) H-8; 6.2 (d, $J_{1',2''}$ = 6.1 Hz, 1H) H-1'; 5.41 (t, $J_{2'',3'}$ = 2.4 Hz and $J_{3',4'}$ = 2.0 Hz) H-3'; 4.54 (m, 1H) H-4'; 4.32 (m, 2H) H-5',5''; 2.54 (dd, $J_{1',2''}$ = 6.1 Hz and $J_{2'',3'}$ = 2.4 Hz) H-2"; 2.29 (s, 3H) N-2-acetyl; 2.13 and 2.09 (two s, 6H) 3' and 5'-O- acetyl. ^{13}C NMR ($CDCl_3$ + CD_3OD): 85.1 (d, J_{CH} = 167.4 Hz) C-1'; 82.5 (d, J_{CH} = 153.9 Hz) C-4'; 74.4 (d, J_{CH} = 157.3 Hz) C-3'; 63.8 (t, J_{CH} = 148.2 Hz) C-5'; 36.4 C-2'. Calc. for $C_{16}H_{18}DN_5O_7$: C, 48.73; H/D, 5.07; N, 17.76. Found: C, 48.82; H/D, 5.18; N, 17.58. MS (FAB^+): calc. for $(M+H)^+$ 395.1426, found 395.1462.

Methyl 2,3-anhydro-5-O-(4-methoxytriphenylmethyl)-β-D-lyxofuranoside (10). Crude 9 (8 g, 54.8 mmol) was treated with 4-methoxytriphenylmethyl chloride (19 g, 61 mmol) as described for 2. The compound did not crystallize and was purified over a short column of silicagel (hexane-dichloromethane 8:2 v/v followed by hexane-dichloromethane 6:4 v/v). Evaporation of the appropriate fraction gives 19.3 g (84%) of 10 as a foam. ^{1}H NMR ($CDCl_3$): 7.24-6.78 (m, 14H) arom.; 4.94 (s, 1H) H-1; 4.01 (t, 1H, $J_{4,5}$ = 6.4 Hz) H-4; 3.74 (d, 1H, $J_{2,3}$ = 3.1 Hz) H-3; 3.72 (s, 3H) methoxy of MMTr; 3.60 (d, 1H) H-2; 3.43 (s, 3H) methoxy; 3.34 (d, 2H) H-5. ^{13}C NMR ($CDCl_3$): 102.4 (d, J_{CH} = 171 Hz) C-1; 75.8 (d, J_{CH} = 152 Hz) C-4; 62.5 (t, J_{CH} = 143 Hz); 56.7 (q) OCH_3; 55.9 (d, J_{CH} = 182 Hz), 55.5 (d, J_{CH} = 182 Hz) C-2 and C-3; 55.2 (q) methoxy of MMTr.

Methyl 2-deoxy-2(R)-deuterio-5-O-(4-methoxytriphenylmethyl)-β-D-threo-pentofuranoside (12a). 10 (10 g, 23.9 mmol) was reduced with lithium aluminum deuteride (2.0 g, 47.8 mmol) according to the procedure described for 2. Short column chromatography on silica gel yields 8.25 g (82%) of pure 12a as a foam. Further elution gives 1.3 g of the 3'-deoxysugar 12b contaminated with 12a. ^{1}H NMR ($CDCl_3$): 7.5-6.8 (m, 14H) arom.; 5.08 (s, 1H) H-1; 4.17 (m, 2H) H-3 and H-4; 3.77 (s, 3H) methoxy of MMTr group; 3.38 (d, 1H, $J_{4,5}$ = 5.4 Hz) H-5,5'; 3.34 (s, 3H) OCH_3; 2.84 (brd, 1H) 3-OH; 2.07 (brs, 1H) H-2'. ^{13}C NMR. 104.9 (d, J_{CH} = 172 Hz) C-1; 83.7 (d, J_{CH} = 148 Hz) C-4; 71.4 (d, J_{CH} = 154 Hz) C-3; 64.0 (t, J_{CH} = 143 Hz) C-5; 54.8 (q) OCH_3; 40.9 (t, J_{CD}= 19 Hz) C-2. MS (FAB^+): $(M+H)^+$ calc. for 422.2078, found 422.2043.

Methyl 2-deoxy-2(R)-deuterio-3-O-methylsulfonyl-5-O-(4-methoxytriphenylmethyl)-β- D-threo-furanoside (13). 12a (8.25 g, 19.6 mmol) was coevaporated with dry pyridine and dissolved in 150 ml of dry pyridine and the solution cooled to 0 °C. Methylsulfonylchloride (3 ml, 39 mmol) was added dropwise and the reaction mixture was kept under stirring at 0 °C for 2 h and at 20 °C for 2 h (alternatively standing at 4 °C overnight). The reaction mixture was then cooled in an ice bath and 150 ml of ice cold saturated aqueous sodium hydrogen carbonate solution was added slowly and

after ca. 1 h the mixture was extracted with dichloromethane (2 x 100 ml). The organic layer was evaporated and coevaporated two times with toluene (below 30 °C) to yield 13 as a foam. yield 9.52 g (97%). A sample was crystallized from methanol. mp. 105-6 °C. ^{1}H NMR ($CDCl_3$): 7.5-6.8 (m, 14H) aromatic protons; 5.21 (dd, 1H, $J_{2,3}$ = 1 Hz, $J_{3,4}$ = 4.7 Hz) H-3; 5.08 (d, 1H, $J_{1,2}$ = 0.5 Hz) H-1; 4.29 (dt, 1H, $J_{4,5}$ = $J_{4,5'}$ = 6.0 Hz) H-4; 3.78 (s, 3H) methoxy of MMTr; 3.49 (dd, 1H, $J_{5,5'}$ = 10 Hz) H-5; 3.33 (s, 3H) OCH_3; 3.24 (dd, 1H) H-5'; 2.80 (s, 3H) mesyl; 2.36 (brs, 1H) H-2'. ^{13}C NMR ($CDCl_3$): 104.4 (d, J_{CH} = 169 Hz) C-1; 80.9 (d, J_{CH} = 146 Hz) C-4; 78.6 (d, J_{CH} = 159 Hz) C-3; 62.7 (t, J_{CH} = 143.5 Hz) C-5; 55.4 (q) OCH_3; 38.7 (t, J_{CD} = 20 Hz) C-2; 38.5 (q) mesyl. Calc. for $C_{27}H_{29}DSO_7$: C, 64.91; H/D, 6.25. Found: C, 64.78; H/D, 6.38.

Methyl 2-deoxy-2(R)-deuterio-3-O-methylsulfonyl-β-D-threo-pento-furanoside (14). Compound 13 (9.25 g, 19 mmol) was treated with 80% aq. acetic acid as described for 5a. The residue was partitionned between water (ca. 150 ml) and toluen (50 ml), the aqeous layer was washed with toluene and evaporated to give an oil wich solidify on standing in a dessicator. Yield 3.27 g (76%). ^{1}H NMR ($CDCl_3$): 5.28 (dd, 1H, $J_{2,3}$ = 1.8 Hz, $J_{3,4}$ = 5 Hz) H-3; 5.08 (d, 1H, $J_{1,2}$ = 1 Hz) H-1; 4.26 (dt, 1H, $J_{4,5}$ = 5.8 Hz) H-4; 3.84 (d, 2H) H-5,5'; 3.39 (s, 3H) OCH_3; 3.10 (s, 3H) mesyl; 2.83 (brs, 1H) 5-OH; 2.32 (brs, 1H) H-2'. ^{13}C NMR ($CDCl_3$): 104.2 (d, J_{CH} = 172 Hz) C-1; 81.6 (d, J_{CH} = 149 Hz) C-4; 78.2 (d, J_{CH} = 159 Hz) C-3; 61.4 (t, J_{CH} = 145.5 Hz) C5; 55.5 (q) OCH_3; 39.4 (t, J_{CD} = 21.5 Hz) C-2; 38.5 (q) mesyl. MS (FAB^-): $(M-H)^-$ calc. for 226.0496, found 226.0517.

Methyl 2-deoxy-2(R)-deuterio-3-O-methylsulfonyl-5-O-toluoyl-β-D-threo-furanoside (15). Compound 14 (3.27 g, 14.4 mmol) was treated with toluoyl chloride (2.8 ml, 21.6 mmol) as described for 5a. After work-up the compound was crystallized from methanol. Yield 2.36 g (92%). mp. 85-86 °C. ^{1}H NMR ($CDCl_3$): 7.95 and 7.23 (m, 4H) toluoyl; 5.34 (m, 1H) H-3; 5.07 (d, 1H, $J_{1,2}$ = 1 Hz) H-1; 4.55 (s, 3H) H-4,5 and 5'; 3.39 (s, 3H) OCH_3; 3.03 (s, 3H) mesyl; 2.40 (s, 4H) H-2' and CH_3 of toluoyl. ^{13}C NMR ($CDCl_3$): 104.4 (d, J_{CH} = 172 Hz) C-1; 78.7 (d, J_{CH} = 147 Hz) C-4; 78.0 (d, J_{CH} = 160.5) C-3; 63.3 (t, J_{CH} = 150.5 Hz) C-5; 55.1 (q) OCH_3; 39.4 (t, J_{CD} = 19 Hz) C-2; 38.3 (q) mesyl. Calc. for $C_{15}H_{19}DSO_7$: C, 52.16; H/D, 6.13; S, 9.28. Found: C, 51.89; H/D, 6.27; S, 9.37.

Methyl 2-deoxy-2(R)-deuterio-3,5-di-O-p-toluoyl-β-D-erythro-pento-furanoside (16). Compound 15 - (3.45 g, 10 mmol) was dissolved in a mixture of dimethylformamide (100 ml) and water (6 ml), the mixture was heated at 130 °C under stirring during the addition of finely powdered sodium-p-toluate (4.7 g, 30 mmol). The reaction mixture was then heated at 100 °C for 48 h and 120 °C for 16 h. The reaction mixture was cooled and filtered. The filtrate was evaporated almost to dryness and water (ca. 100 ml) was added, the precipitate formed was filtered and dissolved in dichloromethane (ca. 150 ml) and the organic layer washed two times with water and evaporated. The residue was evaporated with absolute ethanol and during evaporation the title compound started to crystallise. After cooling at 0 °C the crystals were filtered. Yield 2.74 g (71%). mp. 76-77 °C. ^{1}H NMR ($CDCl_3$): 7.95 and 7.23 (m, 8H) toluoyl; 5.59 (dd, 1H, $J_{2',3}$ = 7.3 Hz, $J_{3,4}$ = 2.7 Hz) H-3; 5.22 (d, 1H, $J_{1,2'}$ = 2.0 Hz) H-1; 4.53 (m, 3H) H-4, 5 and 5'; 3.36 (s, 3H) OCH_3; 2.55 (dd, 1H) H-2'; 2.40 and 2.39 (2s, 6H) CH_3-toluoyl. ^{13}C NMR ($CDCl_3$): 105.5 (d, J_{CH} = 173 Hz) C-1; 81.9 (d, J_{CH} = 147 Hz) C-4; 75.4 (d, J_{CH} = 154 Hz) C-3; 65.1 (t, J_{CH} = 150.5) C-5; 55.1 (q) OCH_3; 39.0 (t, J_{CD} = 20 Hz) C-2. Calc. for $C_{22}H_{23}DO_6$: C, 68.57; H/D, 6.49. Found: C, 68.31; H/D, 6.81. MS (FAB^-): $(M-H)^-$ calc. for 384.1557, found 384.1568.

2-deoxy-2(R)-deuterio-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride (17). Compound 16 (1.46 g, 3.8 mmol) was treated under the same conditions as described for 7a. Yield 1.1 g (74%). mp. 114 °C. ^{1}H NMR ($CDCl_3$): 7.95 (m, 4H) and 7.24 (m, 4H) toluoyl; 6.47 (d, 1H, $J_{1,2}$ = 5.2 Hz) H-1; 5.56 (dd, 1H, $J_{2',3}$ = 7.6 Hz, $J_{3,4}$ = 2.9 Hz) H-3; 4.85 (dt, 1H, $J_{4,5}$ = 3.6 Hz) H-4; 4.64 (m, 2H) H-5,5'; 2.85 (dd, 1H) H-2'; 2.41 (s, 6H) CH_3-toluoyl. ^{13}C NMR ($CDCl_3$): 95.3 (d, J_{CH} = 187.6 Hz) C-1; 84.7 (d, J_{CH} = 151.5) C-4; 73.5 (d, J_{CH} = 155 Hz) C-3; 63.5 (t, J_{CH} = 150.5) C-5; 44.3 (t, J_{CD} = 21 Hz). Calc. for $C_{21}H_{20}DClO_5$: C, 64.69; H/D, 5.64; Cl, 9.11. Found: C, 64.37; H/D, 5.8; Cl, 9.32. MS (FAB^+): $(M-Cl)^+$ calc. for 354.1452, found 354.1472.

1-(2'-Deoxy-2'(R)-deuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione (18b). Thymine (550 mg, 4.4 mmol) was allowed to react with 17 (875 mg, 2.2 mmol) after work up the compound was crystallised from hot ethanol to give 938 mg of 18b (89%). mp. 197 °C. ^{1}H NMR ($CDCl_3$): 9.23 (brs, 1H) NH; 7.95 (m, 4H) toluoyl; 7.32 (m, 5H) toluoyl and H-6; 6.48 (d, 1H, $J_{1',2'}$ = 9 Hz) H-1'; 5.64 (dd, 1H, $J_{2',3'}$ = 6.6 Hz, $J_{3',4'}$ = 1.5 Hz) H-3'; 4.72 (m, 2H) H-5' and 5''; 4.55 (m, 1H) H-4'; 2.42 (s, 6H) CH_3-toluoyl; 2.30 (dd, 1H) dH-2'; 1.61 (s, 3H) 5-CH_3. ^{13}C NMR ($CDCl_3$): 84.8 (d, J_{CH} = 168.5 Hz) C-1', 82.7 (d, J_{CH} = 150.5 Hz) C-4'; 74.9 (d, J_{CH} = 159.5 Hz) C-3'; 64.2 (t, J_{CH} = 148.5 Hz) C-5'; 37.5 (t) C-2'. Calc. for $C_{26}H_{25}DN_2O_7$: C, 65.13; H/D, 5.64; N, 5.84. Found: C, 64.94; H/D, 5.52; N, 5.98. MS (FAB^-): $(M-H)^-$ calc. for 478.1725, found 478.1708.

1-(2'-Deoxy-2'(R)-deuterio-β-D-erythro-pentofuranosyl)-5-methyl-1H,3H-pyrimidine-2,4-dione (19b). Compound 18b (785 mg, 1.64 mmol) was deprotected as described for 19a. Yield 383 mg (96%). mp. 181-184 °C (water). UV (water): λ max = 267 nm (ε = 9800). ^{1}H NMR (CD_3OD): 7.8 (d, 1H, J = 1.1 Hz) H-6; 6.28 (d, 1H, $J_{1',2'}$ = 7.5 Hz) H-1'; 4.41 (dd, 1H, $J_{2',3'}$ = 6.4 Hz, $J_{3',4'}$ = 2.9 Hz) H-3'; 3.94 (m, 1H, $J_{4',5'}$ = 3.2 Hz, $J_{4',5''}$ = 3.8 Hz) H-4'; 3.82 (dd, 1H, $J_{5',5''}$ = 12 Hz) H-5'; 3.74 (dd, 1H) H-5''; 2.22 (t, 1H) H-2'; 1.89 (d, J = 1.2 Hz) 5-CH_3. ^{13}C NMR (CD_3OD): 166.8; 152.3; 138.1; 111.9; 87.9 (d, J_{CH} = 148.5 Hz) C-4'; 85.9 (d, J_{CH} = 169.5) C-1'; 71.6 (d, J_{CH} = 151.5) C-3'; 62.3 (t, J_{CH} = 142.5 Hz); 39.8 (t, J_{CD} = 19 Hz) C-2'; 12.5 (q) 5-CH_3. Calc. for $C_{10}H_{13}DO_5N_2$: C, 49.38; H/D, 6.17; N, 11.52. Found: C, 49.27; H/D, 6.09; N, 11.71. MS (FAB^-): $(M-H)^-$ calc. for 242.0887, found 242.0902.

4-Amino-1-(2'-deoxy-2'(R)-deuterio-β-D-erythro-pentofuranosyl)-1H-pyrimidine-2-one (21b). Cytosine (330 mg, 3 mmol) was condensed with 17 (800 mg, 2.05 mmol) following the procedure described for 18a to afford a mixture of 20b and its α isomer in 95% yield. The mixture was deprotected and separation of isomers carried on as described for 21a. yield 281 mg 63%. mp. 204-6 °C (methanol). UV (water): λ max 271 nm (ε = 9200) pH 7. ^{1}H NMR (CD_3OD): 8.01 (d, 1H, $J_{5,6}$ = 7.5) C-6; 6.25 (d, 1H, $J_{1',2'}$ = 7.0 Hz) H-1'; 5.93 (d, 1H) H-5; 4.38 (dd, 1H, $J_{3',4'}$ = 3.7 Hz, $J_{2',3'}$ = 6.4 Hz) H-3'; 3.96 (m, 1H, $J_{4',5'}$ = 3.3 Hz, $J_{4',5''}$ = 4.0 Hz) H-4'; 3.82 (dd, 1H, $J_{5',5''}$ = 12.1 Hz) H-5'; 3.74 (dd, 1H) H-5''; 2.13 (t, 1H) H-2'. ^{13}C NMR (CD_3OD + D_2O): 167.0; 159.9; 142.3; 96.7; 88.0 (d, J_{CH} = 147 Hz) C-4'; 87.1 (d, J_{CH} =170 Hz) C-1'; 71.6 (d, J_{CH} = 149.5 Hz) C-3'; 62.5 (t, J_{CH} = 142.5 Hz) C-5'; 40.7 (t, J_{CD} = 17 Hz) C-2'. Calc. for $C_9H_{12}DN_3O_4$: C, 47.37; H/D, 6.13; N, 18.42. Found: C, 47.51; H/D, 6.32; N, 18.64. MS (FAB^-): $(M-H)^-$ calc. for 227.0891, found 227.0876.

6-chloro-9(2'-deoxy-2'(R)-deuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pento- furanosyl)purine (22b). 6-chloropurine (516 mg, 3.3 mmol) was condensed with 17 (1.0 g, 2.57 mmol) according to the procedure described for 22a. Crystallized yield (ethanol) 736 mg (56%). mp. 109-10 °C. ^{1}H NMR ($CDCl_3$): 8.66 (s, 1H) H-8; 8.31 (s, 1H) H-2; 7.90 (m, 4H) toluoyl; 7.25 (m, 4H) toluoyl; 6.57 (d, 1H, $J_{1',2'}$ = 8.0 Hz) H-1'; 5.84 (dd, 1H, $J_{2',3'}$ = 6.5 Hz, $J_{3',4'}$ = 1.6 Hz) H-3'; 4.71 (m, 3H) H-4', 5' and 5''; 3.17 (dd, 1H) H-2'; 2.44 (s, 3H), 2.40 (s, 3H) toluoyl-methyl. ^{13}C NMR ($CDCl_3$): 151.6, C-8; 143.4, C-2; 85.1 (d, J_{CH} = 166 Hz) C-1'; 83.1 (d, J_{CH} = 152 Hz) C-4'; 74.7 (d, J_{CH} = 159.5 Hz) C-3'; 63.5 (t, J_{CH} = 149.5 Hz) C-5'; 37.2 (t, J_{CD} = 17 Hz) C-2'. Calc. for $C_{26}H_{22}DClN_4O_5$: C, 61.47; H/D, 4.72; Cl, 6.99; N, 11.03. Found: C, 61.36; H/D, 4.89; Cl, 6.68; N, 10.91. MS (FAB$^+$): $(M+H)^+$ calc. for 508.1498, found 508.1488.

6-Amino-9-(2'-deoxy-2'(R)-deuterio-β-D-erythro-pentofuranosyl)purine (23b). 22b (760 mg, 1.5 - mmol) was treated with methanolic ammonia as described for 22a. Crystallized yield 292 mg (77%). mp. 184 °C. UV (water): λ_{max} = 259 nm (ε= 13600). ^{1}H NMR: 8.33 (s, 1H) H-8; 8.20 (s, 1H) H-2; 6.45 (d, 1H, $J_{1',2'}$ = 8.0 Hz) H-1'; 4.62 (dd, 1H, $J_{2',3'}$ = 5.8 Hz, $J_{3',4'}$ = 2.4 Hz) H-3'; 4.13 (m, 1H, $J_{4',5'}$ = 3.1 Hz, $J_{4',5''}$ = 3.5 Hz) H-4'; 3.87 (dd, 1H, $J_{5',5''}$ = 12.4 Hz) H-5'; 3.78 (dd, 1H) H-5''; 2.79 (dd, 1H) H-2'. ^{13}C NMR: 156.9; 153.2; 149.3; 141.3; 120.3; 89.3 (d, J_{CH} = 149.5 Hz) C-4'; 86.6 (d, J_{CH} = 167.5 Hz) C-1'; 72.7 (d, J_{CH} = 146 Hz) C-3'; 63.6 (t, J_{CH} = 144 Hz) C-5'; 40.7 (t, J_{CD} = 20 Hz) C-2'. Calc. for $C_{10}H_{12}DN_5O_3$: C, 47.62; H/D, 5.55; N, 27.77. Found: C, 47.53; H/D, 5.71; N, 27.9. MS (FAB$^-$): $(M-H)^-$ calc. for 251.1003, found 251.0970.

2-Amino-6-chloro-9-(2'-deoxy-2'-(R)-deuterio-3',5'-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)purine (24b). 2-Amino-6-chloropurine (0.34 g, 2.0 mmol) was con- dense with 17 (723 mg, 1.86 mmol) according to the procedure described for 24a. yield 560 mg (57%); crystallized yield (methanol) 496 mg (51%). mp. 177-8 °C. ^{1}H NMR ($CDCl_3$): 7.90 (m, 5H) toluoyl and H-8; 7.23 (m, 4H) toluoyl; 6.37 (d, 1H, $J_{1',2'}$ = 8.0 Hz) H-1'; 5.80 (dd, $J_{2',3'}$ = 6.2 Hz, $J_{3',4'}$ = 2.1 Hz); 5.35 (bs, 2H) NH_2; 4.68 (m, 3H) H-4', 5' and 5''; 3.13 (dd, 1H) H-2'; 2.43 (s, 3H), 2.39 (s, 3H) toluoyl-methyl. ^{13}C NMR ($CDCl_3$): 140.9, C-8; 85.1 (d, J_{CH} = 167.5) C-1'; 82.9 (d, J_{CH} = 151.5) C-4'; 75.2 (d, J_{CH} = 159.5) C-3'; 64.0 (t, J_{CH} = 149 Hz) C-5'; 36.8 (t, J_{CD} = 18 Hz) C-2'. Calc. for $C_{26}H_{23}DClN_5O_5$: C, 59.71; H/D, 4.78; Cl, 6.79; N, 13.39. Found: C, 59.8; H/D, 5.03; Cl, 6.85; N, 13.24. MS (FAB$^+$): $(M+H)^+$ calc. for 523.1607, found 523.1583.

2-Acetamido-9-(2'-deoxy-2'(R)-deuterio-3',5'-di-O-acetyl-β-D-erythro-pentofuranosyl)-9H-purine -6(1H)-one (26b). Compound 24b (206 mg, 0.39 mmol) was treated as described for 24a. Yield 48 mg (30%). UV (ethanol): λmax = 256 nm (ε = 16000). ^{1}H NMR ($CDCl_3$): 7.78 (s, 1H) H-8; 6.23 (d, 1H, $J_{1',2'}$ = 7.6 Hz) H-1'; 5.42 (dd, 1H, $J_{2',3'}$ = 6.6 Hz, $J_{3',4'}$ = 2.3 Hz) H-3'; 4.50 (m, 1H) H-4'; 4.32 (m, 2H) H-5',5''; 3.0 (t, 1H) H-2'; 2.28 (s, 3H) NHAc; 2.14 (s, 3H) and 2.09 (s, 3H) OAc. ^{13}C NMR ($CDCl_3$): 138.0, C-8; 84.9 (d, J_{CH} = 165 Hz) C-1'; 82.4 (d, J_{CH} = 154 Hz) C-4'; 74.4 (d, J_{CH} = 159.5 Hz) C-3'; 63.9 (t, J_{CH} = 149.5 Hz) C-5'; 36.6 (t, J_{CD} = 20 Hz) C-2'. Calc. for $C_{16}H_{18}DN_5O_7$: C, 48.73; H/D, 5.07; N, 17.76. Found: C, 48.9; H/D, 4.87; N, 17.9. MS (FAB$^+$): $(M+H)^+$ calc. for 395.1426, found 395.1449.

Acknowledgements

Financial supports from the Swedish Board for Technical Development and swedish Natural science - Research Council and the funds from Wallenbergstiftelsen for the purchase of a Jeol 270 MHz FT NMR spectrometer are gratefully acknowledged. We also thank Anders Sandström for the mass spectral measurements and Ingegärd Schiller for secretarial assistance.

References

1. D.R. Kearns, CRC Critical Reviews in Biochemistry 15, 237 (1984).
2. S. Roy, Y. Hiyama, D.A. Torchia and J.S. Cohen, J. Am. Chem. Soc., 108, 1675 (1986).
3. M.J. Robins, H. MacCoss and J.S. Wilson, J. Am. Chem. Soc., 99, 4660 (1977).
4. M.J. Robins, J.S. Wilson and F. Hansske, J. Am. Chem. Soc., 105, 4059 (1983).
5. (a) B. Radatus, M. Yunker and B. Fraser-Reid, J. Am. Chem. Soc., 93, 3086 (1971). (b) B. Fraser-Reid and B. Radatus, ibid, 93, 6342 (1971).
6. S. David and J. Eustache, Carbohyd. Res., 16, 469 (1971) and ibid, 20, 319 (1971).
7. M.Y.H. Wong and G.R. Gray, J. Am. Chem. Soc., 100, 3548 (1978).
8. M. Taniguchi, K. Koga and S. Yamada, Chem. Pharm. Bull., 22, 2318 (1974).
9. F.M. Unger, R. Christian and P. Waldstätten, Carbohyd. Res., 67, 257 (1978).
10. M. Hoffer, Chem. Ber., 93, 2777 (1960).
11. H. Griengl et al., J. Med. Chem., 28, 1679 (1985).
12. Z. Kazimierczuk, H.B. Cottam, G.R. Ravankar and R.K. Robins, J. Am. Chem. Soc., 106, 6379 (1984).
13. (a) R.H. Iwamoto, E.M. Acton and L. Goodman, J. Med. Chem., 6, 684 (1963). (b) W.W. Lee, A.P. Martinez, L. Goodman and D.W. Henry, J. Org. Chem., 37, 2923 (1972).
14. A.G. Beaman and R.K. Robins, J. Appl. Chem., 12, 432 (1962).