Tetrahedron Vol. 49, No. 20, pp. 4373-4382, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Syntheses and Conformational Studies on AZT and its Deuterated Analogues

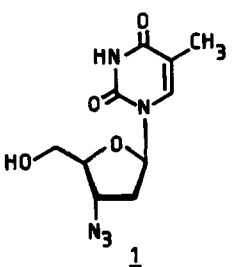
M K Gurjar*, A C Kunwar*, D V Reddy, A Islam, S V S Lalitha, B Jagannadh and A V Rama Rao
Indian Institute of Chemical Technology, Hyderabad 500 007, India

(*Received in UK 2 February* 1993)

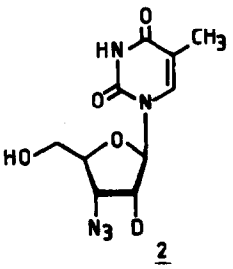
Keywords 3'-Azido-3'-deutero-thymidine, (2'R)-3'-Azido-3'-deoxy-2'-deutero-thymidine, Nuclear magentic resonance, Synthesis, conformation.

Abstract The NMR spectrum of 3'-azido-3'-deoxythymidine (AZT) in aqueous solution provides sum of some of the proton-proton coupling constants. This limitation precludes the determination of the pseudorotational parameters of the sugar ring. Selective deuteration alleviates this problem. The synthesis of 2'-deutero and 3'-deutero- AZT have been described for the first time starting from D-xylose and β-thymidine respectively. NMR study of these analogues in aqueous solutions shows that almost equal amount of C-2'-endo and C-3'-endo species exist in equilibrium.

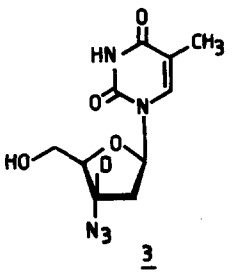
NMR spectroscopy is finding ever increasing applications in the determination of structure and conformations of molecules of interest in biology[1]. The studies in aqueous solvents, because of their similarity to physiological media, permit extension of these results to system in vivo. NMR spectral parameters specially the vicinal coupling constants (J), through Karplus relation[2], are very useful in obtaining the conformations of molecules. However, in special circumstances, arising due to an interplay of the spectral parameters, some spectra only provide sum of the coupling constants and it becomes difficult to derive the structure of the molecules. Deuteration at specific sites results in the change in spectral type and can help in the determination of spectral parameters. We had earlier encountered this problem in connection with our study with 3'-azido-3'-deoxythymidine (1)[3], the main and one of the only three drugs ap-



1



2



3

proved by FDA for the treatment of Acquired Immuno Deficiency Syndrome (AIDS) caused by infection of human immuno-deficiency virus (HIV)[4]. Because of the inability to obtain all the coupling constants individually, we had taken recourse to studies in DMSO-d_6[3,5] which normally provides results similar to those in aqueous solution. In a subsequent study, Plavec et al have investigated AZT in aqueous solution[6,7] and attempted to obtain the structural information from sum of the coupling constants using the graphical method[7]. This analysis, however, failed to provide any definite structural parameter but gave approximate values

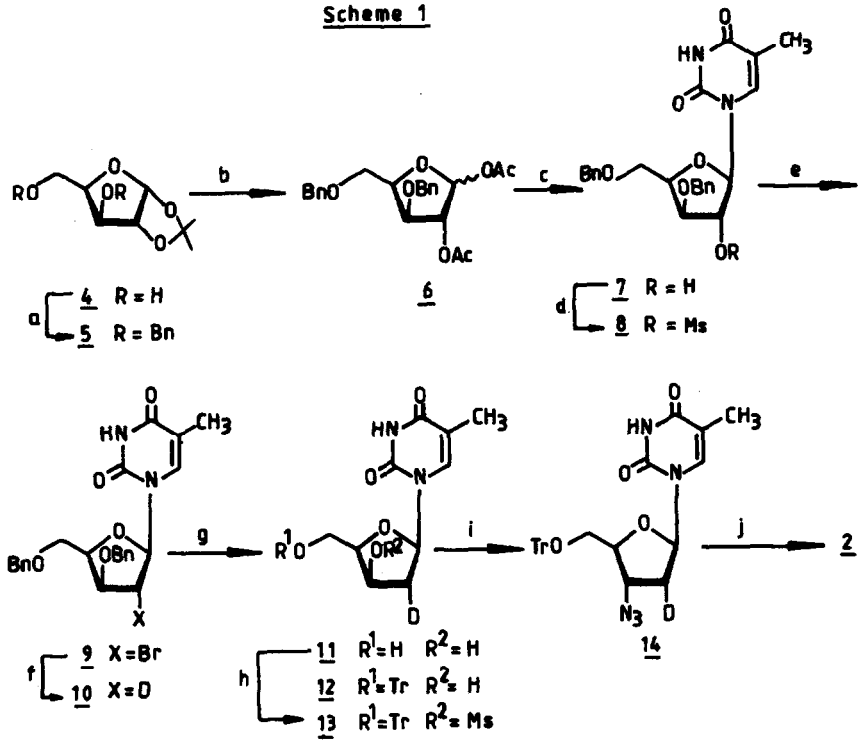
of various conformations present in solution. We believe that this problem can be alleviated by specific deuteration of AZT at 2'-(2) and 3'-position (3). The results of the NMR study of deuterated AZT have been presented in this communication.

Results and Discussions

Synthesis of (2'R)(3'-azido-2',3'-dideoxy-2'-deutero-β-D-ribofuranosyl)-thymine [2'(R)-AZT-2'-d_1] (2)[8]

The known[9] 1,2-O-isopropylidene-α-D-xylofuranose (4) was subjected to benzylation by heating at 80° with BnCl in the presence of powdered KOH for 2h to give the 3,5-di-O-benzylate (5)[10]. Cleavage of the isopropylidene group with 3N H_2SO_4 followed by conventional acetylation produced the dibenzyl-diacetyl derivative (6). Its coupling with thymine followed by removal of acetyl group at 2'-position under Zemplen condition, gave 7[10]. Subsequent mesy-

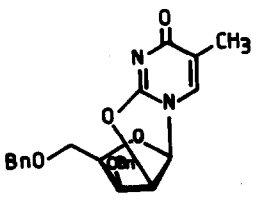
Scheme 1



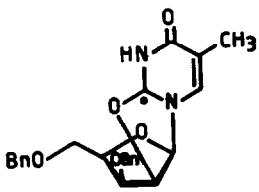
a) BnCl, KOH, Δ, 80°, 2h; b) (i) 3N H_2SO_4, Dioxan, Δ, 0.5h, (ii) Ac_2O, Et_3N, RT, 2h; c) (i) Thymine, HMDS, TMS-Cl, $SnCl_4$, CH_3CN, Δ, 0.5, (ii) NaOMe, MeOH, 1h; d) MsCl, Et_3N, CH_2Cl_2, 1h; e) Py-HBr, Py, Δ, 3h; f) Bu_3SnCl (cat), $NaBD_4$, MeOH, hν, 3h; g) 10% Pd-C, MeOH, H_2 (3 atm), 8h; h) (i) TrCl, Py, 90°, 1h; (ii) MsCl, Py, 1h; i) LiN_3, DMF, 90°, 4h; j) aq. AcOH (80%), 90°, 1h.

lation at C-2' with MsCl-Et_3N in CH_2Cl_2 produced the corresponding mesylate (8). Treatment of 8 with Py-HBr salt in refluxing pyridine afforded the 2'-bromo-derivative (9) (74%) (Scheme 1). In the ^{1}H-NMR spectrum of 9, the characteristic singlet at 6.08 ppm for H-1', suggested[11] the retention of configuration at C-2'. This could perhaps be explained by considering the formation of 2,2'-anhydro intermediate (15).

The removal of bromine with concomitant introduction of deuterium atom by using Corey's reductive dehalogenation was sought[12]. Addition of $NaBD_4$ to the mixture of **9** and Bu_3SnCl (1 molar %) in MeOH irradiated with 500W tungsten lamp, provided the 2'-deuteronucleoside (**10**) in 77% yield. The R configuration at C-2' of **10** was confirmed by ^{1}H-NMR spectrum in which a small coupling constant ($J_{H-1',H-2'}$ 2.6 Hz) for H-1' was observed. The delivery of deuterium radical occuring predominantly from the less hindered α-face was expected[13]. The possibility of forming 2,2'-anhydro radical such as **16** could not be ruled out. The next



15

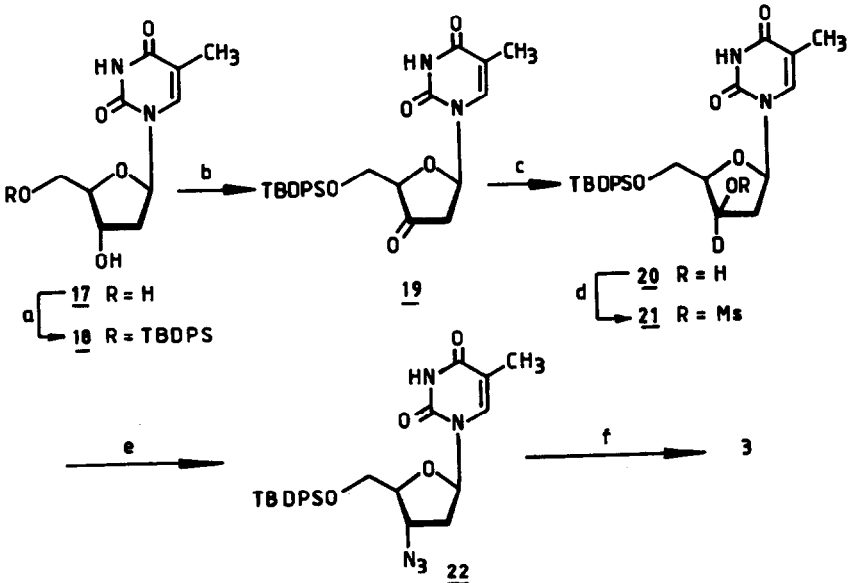


16

set of reactions to introduce the azido group at 3'-position involved the hydrogenolysis of **10** in presence of Pd/C at 45 psi to generate the diol (**11**). Subsequent monotritylation of **11** with 1.1 equivalent of TrCl in pyridine followed by mesylation with MsCl-pyridine gave **13**. Treatment of **13** with LiN_3 in DMF at 90° for 4h effected the formation of the azido derivative **14** which was detritylated with 80% AcOH to afford (2'R)-AZT-2'-d_1 (**2**).

Synthesis of (3'-azido-2',3'-dideoxy-3'-deutero-β-D-ribofuranosyl)thymine (AZT-3'-d_1) (3)

Scheme 2



a) TBDPSCl, DMF, RT, 20h; **b)** Cr_2O_3:Py:Ac_2O, CH_2Cl_2, 45 min; **c)** $NaBD_4$, EtOH, 0°, 2h; **d)** Et_3N, MsCl, CH_2Cl_2, 0°-RT, 1h; **e)** LiN_3, DMF, 90°, 4h; **f)** 1N HCl, MeOH, RT, 8h.

β-Thymidine (**17**) was transformed into the corresponding 3'-ulose derivative (**19**) by successive mono-silylation (**18**) with tert-butyldiphenylsilylchloride (TBDPS-Cl) in the presence of imidazole followed by oxidation with CrO_3-Py-Ac_2O in CH_2Cl_2[14]. Reduction of **19** with $NaBD_4$ gave 3'-deuterated derivative **20** as an exclusive product in 33% overall yield (Scheme 2). The assignment of stereochemistry at C-3' in **20** was based on literature precedents[14] and successive product formation. Treatment of **20** with MsCl-Et_3N in CH_2Cl_2 furnished the 3'-O-mesylate (**21**). **21** was treated with LiN_3 in DMF at 90° for 4h to afford the 3'-azido derivative (**22**) which on desilylation with HCl-MeOH gave the requisite AZT-3'-d_1 (**3**).

NMR Studies

The coupling constants in the sugar ring have been used to determine the conformational preference of the ring by employing the concept of pseudorotation[15]. The two types of pseudorotamers are characterised as the N-type [with angle of pseudorotation -90 < P(N) < 90 and the puckering amplitudie ϕ_m(N)] and S-type [with angle of pseudorotation 90 < P(S) < 270 and the puckering amplitude ϕ_m(S)]. The other important structural parameters which characterize the conformation of nucleosides are the rotational states of sugar-thymine (χ) and sugar-hydroxymethyl (γ).

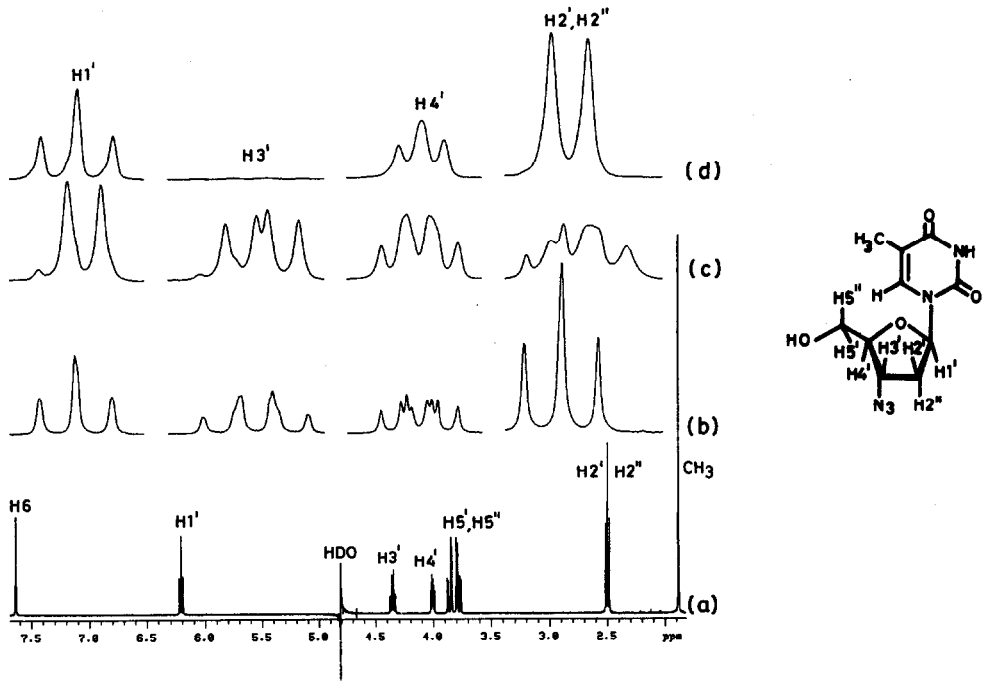


Fig. 1 - a) ^{1}H NMR spectrum of AZT. The glitch around 4.8 ppm is due to suppression of HDO signal. Expansions of H1', H3', H4' and H2', H2" regions of the spectrum: b) for AZT (c) for AZT-2'-d_1 (d) for AZT-3'-d_1.

From the ^{1}H-NMR spectrum of AZT (**1**) (fig.1a), only some of the coupling constants could be obtained individually due to the identical chemical shifts for H-2' and H-2" (for numbe-

ring see fig.1). However the sums $J_{H-1',H-2'} + J_{H-1',H-2''}$ = 13.0 Hz and $J_{H-2',H-3'} + J_{H-2''-H-3'}$ = 12.8 Hz could be obtained accurately. Since the pseudorotational parameters cannot be obtained from this data, the NMR experiments on AZT-2'-d_1 (the extent of deuteration was about 75%) (2) and AZT-3'-d_1 (the extent of deuteration was about 98%) (3) were carried out. Though AZT-2'-d_1 had significant intensity of lines from undeuterated species, they did not interfere in deducing the coupling constants. The coupling constants $J_{H-1',H-2'}$ = 5.9 Hz and $J_{H-2',H-3'}$ = 7.6 Hz were calculated from the spectra. Using the sums of the coupling, from the NMR spectra of AZT (1) and 3'-deutero AZT (3) $J_{H-1',H-2''}$ and $J_{H-2'',H-3'}$ were obtained as 7.1 Hz and 5.2 Hz respectively. The other coupling constants and relevant spectral data have been reported in the Table 1. We were unable to obtain the proton-deuterium couplings possibly due to the rapid quadrupolar relaxation of deuterium which led to the broadening of the resonance lines.

Table 1 : Proton chemical shifts and coupling constants* in AZT and its deutero analogues in D_2O.

Chemical shifts ( ) ¶ (ppm)		Coupling constants (J) (Hz)			
Protons	AZT	Coupling constants	AZT	AZT-2'-d_1	AZT-3'-d_1
H1'	6.216	H1',H2'	a	5.9	a
H2'	2.506	H1',H2''	a	--	a
H2''	2.506	H1',H3'	0.5	--	-
H3'	4.362	H2',H3'	a	7.6	-
H4'	4.015	H2'',H3'	a	--	-
H5'	3.869	H3',H4'	5.5	5.5	-
H5''	3.792	H4',H5'	4.6	4.6	4.6
H6	7.650	H4',H5''	3.5	3.5	3.5
5-methyl	1.887	H5',H5''	-12.6	-12.6	-12.6
		H6,5-methyl	1.2	1.2	1.2

* Chemical shifts have been measured with respect to internal DSS. The accuracy of the chemical shifts is ±0.001 ppm and of the coupling constants ± 0.2 Hz.

¶ The chemical shifts of deuterated analogues are very similar, though show small variations due to isotope shifts. These values differ from those reported earlier[3] (where HDO peak was taken as 4.65 ppm).

a Only the sums $J_{H1',H2'} + J_{H1',H2''}$ = 13.0 Hz and $J_{H2',H3'} + J_{H2'',H3'}$ = 12.8 Hz are obtained.

The pseudorotational analysis performed with PSEUROT[16] showed that the N-type and S-type conformers were in equilibrium. Due to strong correlations between the puckering amplitudes, ϕ_m (N) and ϕ_m(S), they could not be obtained individually. We had to take recourse to the assumption that ϕ_m(N) = ϕ_m(S) in this analysis and the results are presented in Table 2. For a ready comparison, the values obtained in the DMSO-d_6 solution are also included[5]. The correspondence between results in the two solutions is good, though some variations are observed. We find that these variations are outside the experimental errors of the parameters

obtained from the programme PSEUROT. However, the conclusion can be drawn that the N-type and S-type conformers are basically C-3'-endo and C-2'-endo which are present in about equal populations.

Table 2 : Calculated pseudorotational parameters for AZT assuming $\phi_m(N) = \phi_m(S)$.

Solvent	P(N) (o)	P(S) (o)	τ_m(N) (o)	X_N	RMS Error (Hz)
D_2O	12	161	33.2	0.48	0.16
DMSO*	-6	154	33.0	0.43	0.08

* From reference (5).

The coupling constants $J_{H-4',H-5'}$ and $J_{H-4',H-5''}$ provide the conformational preferences of the hydroxymethyl group. Traditionally the assignments for H-5' and H-5" in nucleosides have been made on the basis that the chemical shift of H-5" is smaller than that of H-5'[17] and recently these assignments have been confirmed with experiments on specifically deuterated nucleosides[18]. The assignments for the hydroxymethyl protons have been further confirmed by the NOESY experiments. The fact that $J_{H-4',H-5'}$ and $J_{H-4',H-5''}$ were small, corresponds to predominant g^+ conformer and as expected H-3' showed stronger NOESY peak with high field H-5" compared to low field H-5'. Using simple equations[19,20] we arrived at the populations of the conformers g^+, t and g^- as 58%, 27% and 15% respectively.

The conformation of the sugar ring for AZT in aqueous solution was similar to that for other dideoxynucleosides (ddNs) which showed wide variation in their anti-HIV activity[21]. It was, therefore, difficult to correlate the molecular conformations of the ddNs with their anti-HIV activity. The ddNs must be triphosphorylated (in 3 steps)[22] before they interact with the reverse transcriptase (RT). Therefore, the rate of triphosphorylation (which are different for different ddNs) rather than the molecular conformation of the ddNs, may possibly measure the extent of their anti-HIV activity, though it is widely believed that this step contributes similarly for all the ddNs[23].

Experimental

General remarks: ^{1}H-NMR spectra of AZT, AZT-2'-d_1 and AZT-3'-d_1 were recorded at 400 MHz on a VARIAN UNITY-400 NMR spectrometer. 16-128 Free Induction decays were acquired. Resolution was enhanced by sine-bell multiplication. NOESY experiments were performed on AZT using a mixing time of 1s with a relaxation delay of 2s. The phase sensitive experiment was performed using the procedure of States et al[23]. The data was acquired as a 2x128x 2048 data point matrix with 16 scans per t_1 increment. The data was porcessed as a 512x2048 matrix with Gaussian line broadening.

All other ^{1}H-NMR spectra were recorded on a GEMINI 200 MHz NMR spectrometer. IR spectra were scanned on Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics. MS were recorded on a CEC-21-110B, Finnigan Mat 1210 or MICRO MASS 7070 spectrometers at 70 eV using a direct inlet system. The optical rotations were recorded on DIP

370 JASCO digital polarimeter. All the solvents were distilled before use. Silica gel (60-120) was purchased from ACME Chemical Company, Bombay.

1,2-Di-O-acetyl-3,5-di-O-benzyl-D-xylose (6). A mixture of **4** (11.5 g, 60.52 mmol), BnCl (38.5 ml) and powdered KOH (19.1 g) was heated at 80° for 2h. The reaction mixture was diluted with chloroform and successively washed with water, 1N HCl, brine, dried and concentrated. Purification of the residue by column chromatography using light petroleum-ethyl acetate (10:1) gave **5** (18.78 g, 84%). ^{1}H-NMR data ($CDCl_3$): δ 1.29, 1.42 (2s, 6 H, $(CH_3)_2C$), 3.75 (m, 2 H, CH_2-5), 3.97 (d, 1 H, $J_{H-3,H-4}$ 3.2 Hz, H-3), 4.38 (m, 1 H, H-4), 4.5-4.7 (m, 5 H, 2xPhCH_2 + H-2), 5.93 (d, 1 H, $J_{H-1,H-2}$ 4.5 Hz, H-1), 7.3 (m, 10 H, 2xPh).

Compound **5** (18.75 g, 50.75 mmol) was heated under reflux with dioxan (100 ml) and 3N H_2SO_4 (100 ml). After 30 min. the solution was cooled and neutralised with aqueous NaOH, concentrated to dryness and extracted with ethyl acetate. The organic layer was washed with water, dried and concentrated to give the diol (14.86 g) which was treated with Et_3N (32 ml) and Ac_2O (10.5 ml). After stirring for 2h, 10 ml of methanol was added and the mixture stirred for an additional 30 min, concentrated and dissolved in ethyl acetate. The organic layer was successively washed with 1N HCl, 10% $NaHCO_3$, water, dried and concentrated. The residue was purified by column chromatography on silica gel using light petroleum-ethyl acetate (6:1) as an eluent to afford **6** (16.68 g, 79%), as a thick syrup[9]. ^{1}H-NMR data ($CDCl_3$): δ 2.08, 2.09, 2.13, 2.14 [4s, 6 H, 2xCH_3COO, (α + β)], 5.28 [m, 1 H, H-2, (α + β)], 6.13 (s, 1/2 H, H-1), 6.38 (d, 1/2 H, H-1), 7.3 (m, 10 H, 2xPh).

(2'-Bromo-3',5'-di-O-benzyl-2'-deoxy-β-D-xylofuranosyl)thymine (9). To a solution of **7**[9] (10.65 g, 24.3 mmol) in CH_2Cl_2 (50 ml) at 0° was added Et_3N (7 ml) and MsCl (2.5 ml). After 1h, ice-water (10 ml) was introduced, stirred for 20 min. and then layers were separated. The non-aqueous layer was extracted with 10% $NaHCO_3$ solution, water and dried. Removal of solvent gave a residue which was purified on silica gel with light petroleum-ethyl acetate (7:3) to give **8** (10.4 g, 83%), m.p. 119°-120°, ^{1}H-NMR data ($CDCl_3$): δ 1.67 (s, 3 H, CH_3), 3.25 (s, 3 H, CH_3SO_3), 3.81 (m, 2 H, CH_2-5'), 4.22 (d, 1 H, $J_{H-3',H-4'}$ 3.0 Hz, H-3'), 4.45-4.70 (m, 5 H, 2xPh$\underline{CH_2}$ + H-4'), 5.06 (s, 1 H, H-2'), 5.92 (s, 1 H, H-1'), 7.1-7.45 (m, 11 H, 2xPh + H-6), 10.05 (bs, 1H, NH).

A mixture of **8** (4.12 g, 8.0 mmol), Py-HBr salt (2.88 g, 18.0 mmol) and pyridine (30 ml) were heated under reflux for 3h, cooled to room temperature and poured over ice-water mixture. Conc. HCl was added to neutralise the solution and then extracted with ethyl acetate, which was washed with water, dried and concentrated. The residue was purified on silica gel by eluting with light petroleum-ethyl acetate (4:1) to give **9** (2.96 g, 74%), m.p. 154°-155°, $[\alpha]_D$ -4.6° (c 1.3, chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.51 (s, 3 H, CH_3), 3.66 (m, 2 H, CH_2-5'), 4.08 (bs, 2 H, H-2' + H-3'), 4.42 (m, 5 H, 2xPh$\underline{CH_2}$ + H-4'), 6.08 (s, 1 H, H-1'), 7.2 (m, 11 H, 2xPh + H-6), 8.06 (s, 1 H, NH). Anal. Calcd. for $C_{24}H_{25}BrN_2O_5$: C, 57.5; H, 5.0. Found: C, 57.3; H, 5.1.

(2'R)(3',5'-Di-O-benzyl-2'-deoxy-2'-deutero-β-D-xylofuranosyl)thymine (10). To a solution of **9** (1.0 g, 2.0 mmol), Bu_3SnCl (10 mg) in dry methanol (10 ml) was added $NaBD_4$ (105 mg, 2.49 mmol) in portions while irradiating the reaction mixture with 500W tungsten lamp. After 3h,

the reaction mixture was concentrated and partitioned between water-ethyl acetate. The ethyl acetate layer was washed with 1N HCl, water, dried and concentrated. The residue was purified on silica gel with light petroleum-ethyl acetate (3:1) to give **10** (0.65 g, 77%); $[\alpha]_D$ -18.3° (c 1.3, chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.66 (s, 3 H, CH_3), 2.19 (d, 1 H, $J_{H-1',H-2'}$ 2.6 Hz, H-2'), 3.85 (m, 2 H, CH_2-5'), 4.14 (m, 2 H, H-3' + H-4'), 4.45 (ABq, 2 H, PhCH_2), 4.57 (ABq, 2 H, PhCH_2), 6.23 (d, 1 H, $J_{H-1',H-2'}$ 2.6 Hz, H-1'), 7.25 (m, 10 H, 2xPh), 7.50 (s, 1 H, H-6), 8.95 (bs, 1H, NH).

(2'R)(2'-Deoxy-2'-deutero-3'-O-mesyl-5'-O-trityl- β-D-xylofuranosyl)thymine (13). A solution of **10** (0.65 g, 1.5 mmol), 10% Pd/C (65 mg) in methanol (5 ml) was hydrogenated at 45 psi for 8h. The catalyst was filtered and the filtrate concentrated to give **11** (0.32 g, 85%), m.p. 162°; $[\alpha]_D$ -7.2° (c 1.2, methanol); ^{1}H-NMR data ($CDCl_3$ + CD_3OD): δ 1.90 (s, 3 H, CH_3), 2.03 (d, 1 H, $J_{H-1',H-2'}$ 2.6 Hz, H-2'), 3.92 (m, 3 H, H-4' + CH_2-5'), 4.43 (d, $J_{H-3',H-4'}$ 3.2 Hz, H-3'), 6.10 (d, 1 H, $J_{H-1',H-2'}$ 2.6 Hz, H-1'), 7.88 (s, 1 H, H-6), MS: m/z 243 (M^+).

11 (0.32 g, 1.3 mmol), TrCl (0.44 g, 1.6 mmol) and pyridine (5 ml) were heated at 90° for 1h and then concentrated. The residue was purified on silica gel by eluting with chloroform-methanol (100:1) to give **12** (0.48 g, 77%), which was then treated with pyridine (5 ml) and MsCl (0.5 ml) at room temperature for 1h. Ice-water (2 ml) was added and diluted with chloroform which was successively washed with 1N HCl, $NaHCO_3$ solution, water and dried. On solvent removal, the residue was purified on silica gel by eluting with light petroleum-ethyl acetate (3:1) to give **13** (0.51 g, 91%), $[\alpha]_D$ -25.1° (c 1.1, chloroform); ^{1}H-NMR ($CDCl_3$): δ 1.80 (s, 3 H, CH_3), 2.44 (d, 1 H, $J_{H-1',H-2'}$ 2.3 Hz, H-2'), 2.73 (s, 3 H, CH_3SO_3), 3.35 (dd, 1 H, $J_{H-4',H-5''}$ 7.0 Hz, $J_{H-5',H-5''}$ 10.4 Hz, H-5''), 3.63 (dd, 1 H, $J_{H-4',H-5'}$ 5.8 Hz, $J_{H-5',H-5''}$ 10.4 Hz, H-5'), 4.12 (m, 1 H, H-4'), 5.26 (d, 1 H, $J_{H-3',H-4'}$ 4.6 Hz, H-3'), 6.23 (d, 1 H, $J_{H-1',H-2'}$ 2.3 Hz, H-1'), 7.3 (m, 16 H, 3xPh + H-6), 8.4 (bs, 1 H, NH).

(2'R)-(3'-Azido-2',3'-dideoxy-2'-deutero-β-D-ribofuranosyl)-thymine (2). A stirred solution of **13** (0.51 g, 0.91 mmol), LiN_3 (0.25 g) in DMF (7 ml) was heated at 90° for 4h and then partitioned between water and ether. The ethereal layer was washed with water, dried and concentrated to give a residue, purified by column chromatography on silica gel by using light petroleum-ethyl acetate (7:3) as eluent, to give **14** (0.42 g, 90%), $[\alpha]_D$ + 23.3° (c 1.0, chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.53 (s, 3 H, CH_3), 2.42 (m, 1 H, H-2'), 3.34 (dd, 1 H, $J_{H-4',H-5''}$ 3.4 Hz, $J_{H-5',H-5''}$ 12.8 Hz, H-5''), 3.57 (dd, 1 H, $J_{H-4',H-5'}$ 4.2 Hz, $J_{H-5',H-5''}$ 12.8 Hz, H-5'), 3.98 (m, 1 H, H-3'), 4.36 (m, 1 H, H-4'), 6.24 (d, 1 H, $J_{H-1',H-2'}$ 6.3 Hz, H-1'), 7.3 (m, 15 H, 3xPh), 7.55 (s, 1 H, H-6), 8.02 (bs, 1 H, NH).

14 (0.44 g, 0.86 mmol) and aqueous 80% acetic acid (3 ml) were heated under reflux for 1h and then concentrated. The residue was chromatographed on silica gel by eluting with chloroform-methanol (50:1) to give **2** (0.177 g, 73%), m.p. 114° $[\alpha]_D$ +44.2° (c 1.4, methanol); IR: 2100 cm^{-1} (N_3): CIMS: m/z 269 (M^++1).

(5'-O-tert-Butyldiphenylsilyl-2'-deoxy-3'-deutero-β-D-xylofuranosyl)thymine (20). To a stirring mixture of the complex containing CrO_3 -Py-Ac_2O (30 g, 20 ml, 10 ml) in CH_2Cl_2 (100 ml) was added a solution of **18** (12.0 g, 25.0 mmol) in CH_2Cl_2 (100 ml). After 45 min. the reaction mixture was poured into supernatant ethyl acetate over silica gel, and eluted with ethyl

acetate and concentrated. The residue, after co-distilled with toluene to remove traces of pyridine, was dissolved in dry methanol (100 ml) and cooled to 0°, $NaBD_4$ (0.5 g, 13.4 mmol) was added to the methanolic solution with stirring and after 2h the reaction mixture was concentrated and partitioned between water and ethyl acetate. The ethyl acetate layer was washed with 20% CH_3COOH, $NaHCO_3$ solution, water and dried. Evaporation of solvent afforded a residue which was purified on silica gel by eluting with light petroleum-ethyl acetate (4:1) to give **20** (4.0 g, 33%), m.p. 91°; $[\alpha]_D$ +15.0° (c 1.1, chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.06 (s, 9 H, $(CH_3)_3C$), 1.57 (s, 3 H, CH_3), 2.06 (dd, 1 H, $J_{H-1',H-2'}$ 4.0 Hz, $J_{H-2',H-2''}$ 15.6 Hz, H-2'), 2.60 (dd, 1 H, $J_{H-1',H-2''}$ 8.8 Hz, $J_{H-2',H-2''}$ 15.6 Hz, H-2''), 3.82 (t, 1 H, $J_{H-4',H-5'} = J_{H-4',H-5''}$ 6.6 Hz, H-4'), 4.06 (m, 2 H, CH_2-5'), 6.22 (dd, 1 H, $J_{H-1',H-2'}$ 4.0 Hz, $J_{H-1',H-2''}$ 8.8 Hz, H-1'), 7.3-7.8 (m, 11 H, 2xPh + H-6), 8.17 (bs, 1 H, NH). Anal. Calcd. for $C_{26}H_{31}DN_2O_5Si$: C, 64.8; H/D 6.9. Found: C, 64.7; H, 6.6.

(3'-Azido-5'-O-tert-butyldiphenylsilyl-2',3'-dideoxy-3'-deutero- β -D-ribofuranosyl)thymine (22). To a solution of **20** (4.0 g, 8.32 mmol), Et_3N (3.6 ml) in CH_2Cl_2 (50 ml) was added MsCl (2.0 ml) at 0°. After 1h at room temperature, ice-water (10 ml) was added and the layers separated. The non-aqueous layer was washed with 5% citric acid, $NaHCO_3$ solution, water, dried and concentrated. The residue was purified by column chromatography on silica gel by eluting with light petroleum-ethyl acetate (4:1) to give **21** (3.8 g, 82%). ^{1}H-NMR data ($CDCl_3$): δ 1.15 (s, 9 H, $(CH_3)_3C$), 1.67 (s, 3 H, CH_3), 2.52 (dd, 1 H, $J_{H-1',H-2'}$ 4.5 Hz, $J_{H-2',H-2''}$ 16.4 Hz, H-2'), 2.92 (dd, 1 H, $J_{H-1',H-2''}$ 8.0 Hz, $J_{H-2',H-2''}$ 16.4 Hz, H-2''), 3.65 (s, 3 H, CH_3SO_3), 4.17 (m, 3 H, H-4' + CH_2-5'), 6.27 (dd, 1 H, $J_{H-1',H-2'}$ 4.5 Hz, $J_{H-1',H-2''}$ 8.0 Hz, H-1'), 7.3-7.8 (m, 11 H, 2xPh + H-6), 8.20 (bs, 1 H, NH).

A solution of **21** (3.5 g, 6.26 mmol), LiN_3 (1.0 g, 20.8 mmol) in DMF (10 ml) was stirred for 4h at 90° and then partitioned between water and ether. The ethereal layer was washed with water, dried and concentrated to give a residue purified by column chromatography on silica gel by using light petroleum-ethyl acetate (6:1) as eluent to give **22** (2.8 g, 88%), $[\alpha]_D$ + 45.4° (c 0.6 chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.10 (s, 9 H, $(CH_3)_3C$), 1.65 (s, 3 H, CH_3), 2.30 (dd, 1 H, $J_{H-1',H-2'}$ 6.2 Hz, $J_{H-2',H-2''}$ 12.9 Hz, H-2'), 2.47 (dd, 1 H, $J_{H-1',H-2''}$ 6.2 Hz, $J_{H-2',H-2''}$ 12.9 Hz, H-2''), 3.75-4.1 (m, 3 H, H-4' + CH_2-5'), 6.27 (t, 1 H, $J_{H-1',H-2'} = J_{H-1',H-2''}$ = 6.2 Hz, H-1'), 7.2-7.8 (m, 11 H, 2xPh + H-6), 9.10 (bs, 1 H, NH).

(3'-Azido-2',3'-dideoxy-3'-deutero- β -D-ribofuranosyl)thymine (3). **22** (1.8 g, 3.55 mmol) and 1N HCl (1.2 ml) in methanol (25 ml) were stirred at room temperature for 8h and then neutralised by adding solid $NaHCO_3$. The solvent was evaporated, residue extracted with ethyl acetate and dried. Solvent removal furnished a product which was purified on silica gel column with chloroform-methanol (50:1) to give **3** (0.54 g, 57%); m.p. 115°; $[\alpha]_D$ + 55.4° (c 1.3, methanol). MS: m/z 268 (M^+).

References

1. Wuthrich, K.; "NMR of Proteins and Nucleic Acids", John Wiley & Sons, New York (1986).
2. Haasnoot, C.A.G.; de Leeuw, F.A.A.M. and Altona, C.; Tetrahedron, **36** (1980) 2783.

3. Swapna, G.V.T.; Jagannadh, B.; Gurjar, M.K.; and Kunwar, A.C. Biochem. Biophys. Res. Commun., **164** (1989), 1086.
4. De Clerq, F.; Trends Pharmacol. Sci., **11** (1990) 199.
5. Jagannadh, B.; Reddy, D.V.; and Kunwar, A.C.; Biochem. Biophys. Res. Commun., **176** (1991) 386.
6. Plavec, J.C.; Koole, L.H.; Sandstrom, A.; and Chattopadhyaya, J. Tetrahedron, **35** (1991) 7363.
7. Rinkel, L.I.; Altona, C.; J. Biomol. Struct. Dyn. **4** (1987) 621.
8. For preliminary communication, see - Gurjar, M.K.; Lalitha, S.V.S.; Sharma, P.A.; and Rama Rao, A.V.; Tet. Lett., **33** (1992) 7945.
9. Baker, B.R.; Schuab, R.E.; J. Am. Chem. Soc., **77** (1955) 5900.
10. Benhaddou, R.; Czernecki, S.; Valery, J.M.; Bellosta, V.; Bull. Chem. Fr.; **127** (1991) 108.
11. Hrebabecky, H.; Holy, A.; Carbohydr. Res. **216** (1991) 179.
12. Corey, E.J.; Suggs, J.W.; J. Org. Chem.; **40** (1975) 2254.
13. Hartwig, W.; Tetrahedron, **39** (1983) 2609.
14. Seela, F.; Heinz, P.M.; Helv. Chem. Acta, **74** (1991), 1081.
15. Altona, C.; and Sundaralingam, M.; J. Am. Chem. Soc. **94** (1972), 8205.
16. de Leeuw, F.A.A.M.; and Altona, C.; Quant. Chem. Progr. Exch., No.463, University of Indiana at Bloomington (1983).
17. Cheng, D.M.; Kan, L.S.; Frechet, D.; Tso, P.O.P.; Vesugi, S.; Shida, T.; and Ikehara, M.; Biopolymers, **23** (1984), 775.
18. Kline, P.C.; and Seriani, A.S.; Magn. Reson. Chem.; **28** (1990), 324.
19. Kunwar, A.C.; Jagannadh, B.; and Reddy, D.V.; in "Magnetic Resonance Current Trends", Eds. Khetrapal, C.L.; and Govil, G.; Narosa Publishing House, New Delhi, 1991, p171.
20. Dhingra, M.; and Sharma, R.H.; in "Stereodynamics of Molecular Systems, Eds. Sharma, R.H.; Adenine Press, New York, 1979, p3.
21. DeClerq, E.; Antiviral Res.; **12** (1989) p1.
22. Hao, Z.; Cooneq, D.A.; Hartman, N.R.; Brode, S.; and Johns, D.G.; Mol. Pharmacol., **34** (1988), 431.
23. States, D.J.; Haberkom, R.A.; and Ruben, D.J.; J. Magn. Reson. **48** (1982), 286.

IICT Communication No 3169