Tetrahedron Vol 44, No 15, pp 4895 to 4903, 1988
Printed in Great Britain.

0040-4020/88 $3.00 + .00

SYNTHESIS OF 3'-C-CYANO-3'-DEOXY-PENTOFURANOSYLTHYMINE NUCLEOSIDES

Ana Calvo-Mateo, María-José Camarasa, Angel Díaz-Ortíz and Federico G. De las Heras*

Instituto de Química Médica, Juan de la Cierva 3, 28006-Madrid, Spain.

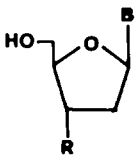
Antonio Alemany

Instituto de Química Orgánica General, Juan de la Cierva 3, 28006-Madrid, Spain.

(*Received in UK 26 April 1988*)

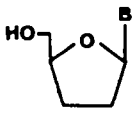
Abstract - A series of 3'-C-cyano-3'-deoxy-pentafuranosylthymine nucleosides have been synthesized from 1-(β-D-ribofuranosyl)thymine (7). 2',5'-Selective protection of 7 followed by oxidation afforded the corresponding 3'-keto-nucleoside (9). The latter reacted with NaCN to give a mixture of 3'-cyano-hydrins which were 3'-deoxygenated to afford 1- 2',5'-di-O-(t-butyldimethyl-silyl)-3'-C-cyano-3'-deoxy-β-D-xylo-pentofuranosyl thymine (14). Deprotection of 14 and selective 5'-O-protection gave the 5'-O-(t-butyldimethylsilyl) derivative 16 which by 2'-deoxygenation yielded the corresponding 3'-C-cyano-2', 3'-dideoxy-β-D-threo nucleoside 22. Reaction of 16 with an acylating reagent in the presence of a strong base gave the 3'-C-cyano-2',3'-dideoxy-2',3'-didehydro nucleoside 12. Epimerization of 14 and 22 with NaOH afforded 3'-C-cyano-3'-deoxy-β-D-ribo (18) and 3'-C-cyano-3'-deoxy-β-D-erythro (24) nucleosides, respectively. Reaction 12, 22 and 24 with HCl gave the correspon ding deprotected nucleosides 13, 23 and 25. 5-O-Acetyl-3-C-cyano-3-deoxy-1,2 -O-isopropylidene-α-D-ribofuranose has also been prepared by a route similar to that described for 22.

2',3'-Dideoxynucleosides are among the most potent and selective anti-HIV agents deacribred so far.[1,2,3] Some compounds of this group such as 3'-azido-3'-deoxythymidine (AZT, 1), and 2',3' dideoxycytidine (4), are currently under clinical trials.[4] Other related compounds, namely, 2',3'-dideoxythymidine (3), 2',3'-dideoxy-2',3'-didehydrothymidine (5), 2',3'-dideoxy-2',3'-didehydrocytidine (6), 2',3'-dideoxy-3'-fluorocytidine (2), also show potent and selective inhibition of HIV in vitro.[1,2,3] The accepted mechanism of action for AZT, and other 2',3'-dideoxynucleosides, involves the non selective phosphorylation by cellular kinases to the 5'-triphosphate (AzddTTP). This may act as a competivive inhibitor or an alternate substrate of HIV reverse transcriptase. Incorporation or AzddTTP into DNA, produces chain termination. AzdtTTP inhibits HIV reverse transcriptase one hundred times better than cellular DNA polymerase and is also able to cross the blood-brain barrier.[5] However it also shows bone marrow suppresion and other side effects which make necessary the search for new nucleoside analogues more selective and less toxic in its anti-AIDS activity.



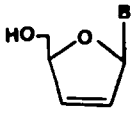
1, (AZT). R = N_3, B = thymin-1-yl

2, R = F, B = cytosin-1-yl



3, B = thymin-1-yl

4, B = cytosin-1-yl



5, B = thymin-1-yl

6, B = cytosin-1-yl

A group of choice candidates are the 3'-C-cyano-3'-deoxynucleosides. The main methods for the introduction of C-cyano branches in carbohydrates have been briefly reviewed.[6] Two of these methods have been recently used for the synthesis of 3'-C-cyanonucleosides. 3'-C-cyano-3'-deoxythymidine and its α-anomer have been prepared by reaction of a methyl 3-C-cyano-2,3-dideoxy-α-D-erythro-pentofuranoside with thymidine.[7] 1-(3'-C-Cyano-3'-deoxy-β-D-arabinofuranosyl)uracil has been synthesized by reaction of a 1-(2',3'-anhydro-β-D-lyxofuranosyl)uracil with sodium cyanide.[8] In a preliminary account of this work, we have also reported the synthesis of 3'-C-cyano-3'-deoxythymidine derivatives by reaction of sodium cyanide with a 3'-ketothymidine nucleoside.[9]

In this paper we report the synthesis of 3'-C-cyano-3'-deoxy derivatives of β-D-xylo- (15), β-D-ribo- (18), 2'-deoxy-β-D-threo- (24) and 2'-deoxy-β-D-erythro-pentofuranosylthymine (26), as well as the 1-(3'-C-cyano-2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)thymine (13).

Reaction of 1-(β-D-ribofuranosyl)thymine (7)[10] with 3 equivalents of t-butyldimethylsilyl chloride in pyridine afforded the 2',5'-di-O-t-butyldimethylsilyl derivative 8 in 68% yield. The latter was reacted with CrO_3/Pyridine/Ac_2O[11] to give the 1-(β-D-erythro-pentofuranos-3-ulosyl)thymine (9) in 73% yield. Reaction of 9 with sodium cyanide and sodium bicarbonate in an ethyl ether-water, two phases system, afforded stereoselectively a (12:1) mixture of the two cyanohydrin epimers 10 in 85% total yield. These compounds, which partially decomposed to ketonucleoside 9, on standing in solution at room temperature were 3'-deoxygenated[12] by reaction with phenyloxythiocarbonyl chloride and 4-dimethylaminopyridine.[13] The resulting (6:1) mixture of 3'-C-cyano-3'-O-(phenyloxythiocarbonyl)epimers 11 was reacted with tributyltin hydride in the presence of azobisisobutironitrile to give stereoselectively 1-(3'-C-cyano-3'-deoxyxylofuranosyl)-thymine 14 (60% yield from 9), as the only product. Removal of the 2'- and 5'-O-(t-butyldimethylsilyl) protecting groups with tetrabutylammonium fluoride in THF afforded 15 in 61% yield.

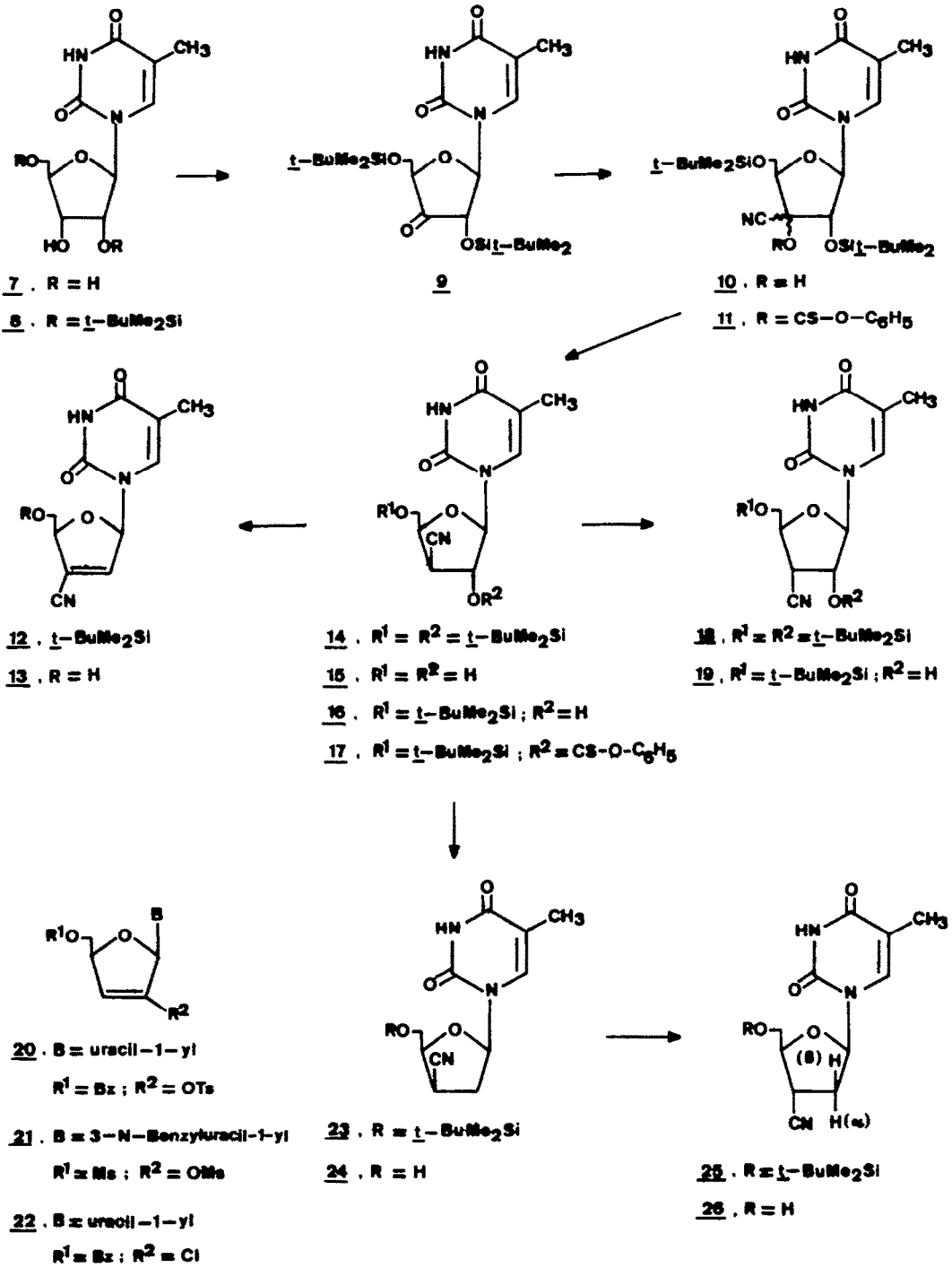
The β-D-xylo derivative 15, was 2'-deoxygenated to the β-D-threo nucleoside 24. Reaction of 15 with 1.1 equivalents of t-butyldimethylsilyl chloride gave the selectively 5'-O-protected nucleoside 16 (93%). Treatment of the latter with phenyloxythiocarbonyl chloride and pyridine in methylene chloride, followed by reaction of the resulting thiocarbonate 17 with tributyltin hydride and α,α'-azobisisobutironitrile in toluene gave 23 (60% yield). Deprotection of 23 with 0.1N HCl afforded 24 in 94% yield.

Reaction of 16 with phenyloxythiocarbonyl chloride in the presence of 4-dimethylamino-pyridine, a stronger base than the pyridine used before, did not afford the expected 2'-O-thiocarbonyl derivative 17 but the 3'-C-cyano-2',3'-unsaturated nucleoside 12 in 80% yield. This compound was deprotected with a 0.1 N solution of HCl in methanol to give 13 in 85% yield. The 2',3'-double bond of 12 and 13 was demonstrated by analytical and spectroscopic data. The IR spectrum showed a band at 2215 cm^{-1} characteristic of a conjugated nitrile . The ^1H NMR spectrum showed the absence of signals corresponding to 2'-CH_2 and H-3' and the presence of an olefinic H-2' at 7.08 ppm. However, the value of the allylic coupling constant $J_{2',4'}$= 4.3 Hz was much higher than expected ($J_{2',4'}$= 1.5 Hz).[14,15] The coupling constants of the pent-2'-enofuranosyl ring of 12 and 13 ($J_{1',2'}$= 1.5-1.8 Hz; $J_{1',4'}$= 1.6-2.0 Hz; $J_{2',4'}$= 3.9-4.3 Hz) were very close to those reported for 2'-substituted-2',3'-unsaturated nucleosides 20[16], 21[17] and 22[18] ($J_{1',3'}$= 1-1.8 Hz; $J_{1',4'}$= 1.6-1.8 Hz; $J_{3',4'}$= 3.2-4.0 Hz). This prompted us to check whether the CN group moved from 3' to 2' during the latter reaction. NOE experiments[19] (Table 1) carried out with 12 showed that irradiation of the olefinic proton at 7.08 ppm produced a much higher enhancement to the signal of H-1' than to the signal of H-4'. This indicates that the olefinic proton is H-2' and, thus, that the cyano group in 12 and 13 is at C-3'.

The 3'-C-cyano-β-D-xylo (14) and β-D-threo (23) nucleosides were transformed to the corresponding 3'-C-cyano-β-D-ribo (18) and β-D-erythro (25) epimers under basic conditions. The most efficient epimerizations in terms of yield (70-75%) and reaction time were carried out by treatment of a methanolic solution of the "cyano up" compounds 14 and 23 with a solution of NaOH in methanol up to pH 9 and refluxing of the resulting mixture. Good yields (75%) of the "cyano down" compounds 18 and 25 were also obtained by reaction of 14 and 23 with a pH 9 refluxing

solution of tetrabutylammonium hydroxide (TBAH) in methanol/chloroform. With Lower values of pH and temperatures the epimerization was very slow or did not take place at all.

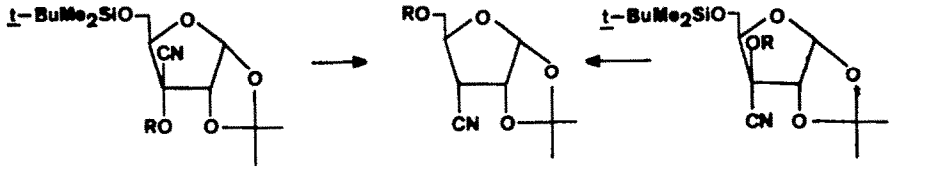
The work up of these reactions involved treatment with 0.1 N HCl to neutralise the base (NaOH, TBAH). The use of more concentrated HCl afforded in some cases the 5'-O-deprotected 8-D-ribo (19) and 8-D-erythro (26) compounds as minor side products.



SCHEME 1

The stereochemistry at the C-3' position of cyanohydrins 10 and thiocarbonates 11 has not been determined, and cannot be inferred from the orientation of the 3'-C-cyano group of the nucleoside 14 resulting from 3'-deoxygenation.[12] The stereochemistry of the cyano group of 14 depends on the relative steric hindrance of the α and β faces of the furanose ring which facilitates the approach of the tributyltin hydride from the less hindered side of the molecule.[12]

This is supported by the transformations 27,31 → 30 described in Scheme 2. The two cyanohydrin epimers 27 and 31, the configuration at C-3 of which have been unequivocally determined,[21] were deoxygenated by reaction with phenyloxythiocarbonyl chloride and then with tributyltin hydride as described before. Both 27 and 31 afforded the same 3-C-cyano-3-deoxyribofuranose 29, as the only product, which was identified as the 5-O-acetate 30. In this case, the hydrogen atom enters from the less hindered, β-face of the molecule, opposite to the bulky 1,2-O-isopropylidene group.[20] In the case of cyanohydrin nucleosides 10, the hydrogen enters from the α-face, to afford the kinetically controlled 3'-C-cyano-β-D-xylo nucleoside 14. This suggests that the α-face, opposite to the thymine and the 5'-O-substituent, is the less hindered face of the present nucleoside furanosyl ring. This is in agreement with the way in wich epimerizations 14 → 18 and 23 → 25 take place. The thermodynamically more stable 3'-C-cyano epimers (18 and 25) are those in which the cyano group is in the less hindered α-face of the nucleoside furanosyl ring.



27 , R = H
28 , R = CS–O–C6H5
29 , R = t-BuMe2Si
30 , R = Ac
31 , R = H
32 , R = CS–O–C6H5

SCHEME 2

Cyanosugar 30 can be a suitable synthetic intermediate for the synthesis of 3'-C-cyano-3'-deoxy-β-D-ribo-pentofuranosyl nucleosides, such as 18. The reaction of suitably protected derivatives of 30 with nucleic acid bases should provide a synthetic route to the above nucleosides alternative to that described here.

Table 1. NOE values for 12, 24, and 26

Compound	Proton Irradiated	NOEs observed at the indicated protons							
		H-1'	H-2'(α)	H-2'(β)	H-3'	H-4'	H-5'	H-6	5'-OH
12	H-1'	-65	2.5					1.0	
	H-2'	2.9	-64			0.7			
	H-4'		0.8			-77	5.6		
24	H-1'	-90	6.0		2.5	3.3		1.8	
	H-2'(α)	7.6	-60	14.0	2.5				
	H-2'(β)	1.3	12	-66				6.2	
	H-3'*(H-5')	2.5	5.0	0.5	-79	18.1	*	2.1	11.5
26	H-1'	-74	6.5			2.9		3.3	
	H-3'			2.6	-74		5	2.6	

* The chemical shifts of H-3' and H-5' are coincident in $(CD_3)_2SO$ solution. Thus, the NOEs induced by the three protons are observed upon irradiation if H-3' (H-5').

The stereochemistries at C-3' for nucleosides **23** and **25** and thus, for the β-D-xylo (14-17) and β-D-ribo (18 and 19) derivatives has been unequivocally determined by NOE experiments.[19] The data shown in Table 1 indicate that in compound β-D-threo **23**, irradiation of H-1', induce a NOE to H-2' (α), H-3' and H-4', irradiation of H-3' induce a NOE to H-1', H-2'(α) and H-4', and irradiation of H-2' (α) and H-2'(β) confirm the above NOE pattern. This demonstrates that protons H-1', H-2' (α), H-3' and H-4' are all in the α-face of the furanose ring. For compound β-D-erythro **25**, irradiation of H-1' induces a NOE to H-2'(α) and H-4', while, irradiation of H-3' induces a NOE to H-2'(β) and H-5'. This demonstrates that H-1' is in the α-face and H-3' in the β-face of the furanose ring and confirm the proposed structures.

EXPERIMENTAL

M.ps were measured with a kofler hot-stage apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AM-200 or a Varian XL-300 spectrometer using Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrometer. Mass spectra were obtained with a Vacuum Generators VG 12-250 spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Analytical tlc plates were purchased from Merck. Preparative tlc was performed on glass plates coated with a 2mm layer of silica gel PF_{254} (Merck). Flash column chromatography was performed with silica gel 60 230-400 mesh (Merck). Compounds were detected by UV light (254 nm) or by spraying the plates with 30% H_2SO_4 in ethanol, and heating.

1-[2',5'-Di-O-(t-butyldimethylsilyl)-β-D-ribofuranosyl]thymine (**8**). A mixture of **7** (1.29 g, 5 mmol), dry pyridine (10 mL) and t-butyldimethylsilyl chloride (2.26 g, 15 mmol) was stirred at room temp. for 48 h. The solvents were evaporated to dryness and the residue dissolved in chloroform was washed with cold (4 C) 1 N HCl (50 mL) and water (2 x 50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography using hexane/ethyl acetate (4:1) as eluent to give **8** (1.65 g, 68%) as a white foam; λ_{max} (EtOH) 265 nm (ε, 8820); ^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.86, 0.93 (2s, 18H, t-Bu), 1.90 (s, 3H, 5-CH_3), 2.66 (bs, 1H, 3'-OH), 3.70-4.30 (m, 5H, H-2', H-3', H-4', H-5'), 6.03 (d, 1H, $J_{1',2'}$ 5.5 Hz, H-1'), 7.50 (s, 1H, H-6), 9.40 (bs, 1H, 3-NH). Anal. Calcd. for $C_{22}H_{42}N_2O_6Si_2$: C, 54.28; H, 8.70; N, 5.75. Found: C, 54.07; H, 8.65; N, 5.81.

1-[2',5'-di-O-(t-butyldimethylsilyl)-β-D-erythro-pentofuranos-3'-ulosyl]thymine (**9**). Compound **8** (0.96 g, 2 mmol) was added to a previously prepared solution of CrO_3 (0.6 g, 6 mmol), pyridine (1 mL, 12 mmol), acetic anhydride (0.6 mL, 6 mmol) and methylene chloride (14 mL). The resulting mixture was stirred at room temp for 45 min, evaporated to dryness, and the residue, suspended in ethyl acetate (10 mL), was filtered through a silica gel (20 g) column using ethyl acetate as eluent. The filtered solution was evaporated to dryness and the residue coevaporated with ethanol (3 x 5 mL). The final residue was chromatographed (coloumn) with hexane/ethyl acetate (5:1) to give **9** (0.70 g, 73%) as a white foam; $[\alpha]_D$ + 72° (c 0.5, $CHCl_3$); ν_{max} (Nujol) 1780 (furanosulose C=O), 1700 cm^{-1} (thymine C=O); ^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.82, 0.90 (2s, 18H, t-Bu), 1.93 (s, 3H, 5-CH_3), 3.90 (m, 2H, H-5'), 4.20 (d, 1H, $J_{1',2'}$ 8 Hz, H-2'), 4.23 (m, 1H, H-4'), 6.27 (d, 1H, H-1'), 7.50 (s, 1H, H-6), 9.40 (bs, 1H, 3-NH). Anal. Calcd. for $C_{22}H_{40}N_2O_6Si_2$: C, 54.51; H, 8.32; N, 5.78. Found: C, 54.20; H, 8.47; N, 5.92.

5'-O-(t-Butyldimethylsilyl)-3'-C-cyano-3'-deoxy-2',3'-didehydro thymidine (**12**). A mixture of **16** (0.38 g, 1 mmol), acetonitrile (15 mL), 4-dimethylaminopyridine (0.25 g, 2 mmol) and phenyloxythiocarbonyl chloride (0.2 mL, 1.1 mmol) was stirred at room temperature for 1 h. The solvents were evaporated to dryness and the residue was purified by column chromatography with ethyl acetate/hexane (1:1) as the eluent and crystallized from chloroform/hexane to afford **12** (0.29 g, 80%) m.p. 128-9°C; ν_{max} (KBr) 2215 cm^{-1} (CN); λ_{max} (EtOH) 260 nm (ε 13150); ^{1}H NMR ($CDCl_3$, 200 MHz): δ0.93 (s, 9H, t-Bu), 1.90 (q, 3H, J 1.2 Hz, 5-CH_3), 4.0 (m, 2H, H-5'), 4.96 (m, 1H, H-4'), 6.66 (dd, 1H, $J_{1',2'}$ 1.8, $J_{1',4'}$ 2.1 Hz, H-1'), 7.08 (dd, $J_{2',4'}$ 4.3 Hz, H-2'), 7.30, (q, 1H, H-6), 9.65 (bs, 1H, 3-NH). Anal. Calcd. for $C_{17}H_{25}N_3O_4Si$: C, 56.17; H, 6.93; N, 11.56. Found: C, 56.35; H, 6.70; N, 11.44.

3'-C-Cyano-3'-deoxy-2',3'-didehydrothymidine (13). Compound 12 (0.36 g, 1 mmol) was stirred at room temp with a 0.1 N solution of HCl in methanol for 30 min. The reaction mixture was neutralized with a 1N solution of NaOH in methanol and the solvent was evaporated to dryness. The residue was crystallized from ethyl acetate to give 13 (0.21 g, 85%); m.p. 200-1°C; ν_{max} (KBr) 2210 cm^{-1} (CN); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ1.74 (d, 1H, J 1.3 Hz, 5-CH_3), 3.72 (bs, 2H, H-5'), 5.01 (m, 1H, H-4'), 5.41 (t, $J_{5',OH}$ 4.4 Hz, 5'-OH), 6.99 (dd, 1H, $J_{1',2'}$ 1.5, $J_{2',4'}$ 3.9 Hz, H-2'), 7.14 (t, 1H, $J_{1',4'}$ 1.9 Hz, H-1'), 7.65 (q, 1H, H-6), 11.44 (bs, 1H, 3-NH). Anal. Calcd. for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.49; N, 16.86. Found: C, 52.88; H, 4.51; N, 16.81.

1- [2',5'-Di-O-(t-butyldimethylsilyl)-3'-C-cyano-3'-deoxy-β-D-xylo-pentofuranosyl] thymine (14). A mixture of 9 (0.48 g, 1 mmol), ethyl ether (8 mL), water (4 mL), sodium bicarbonate (0.16 g, 2 mmol) and sodium cyanide (0.05 g, 1 mmol) was vigorously stirred at 15°C for 16 h: the organic phase was separated, and the aqueous phase was washed with ethyl ether (2 x 8 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue, dissolved in acetonitrile (10 mL), reacted with 4-dimethylaminopyridine (0.25 g, 2 mmol) and phenyloxythiocarbonyl chloride (0.2 mL, 1.1 mmol) at room temp for 2 h. The solvent was evaporated to dryness, and the residue was purified by preparative tlc with hexane/ethyl acetate (5:1) as the eluent to yield a mixture of the two epimeric 1-[2',5'-di-O-(t-butyldimethylsilyl)-3'-C-cyano-3'-O-(phenyloxythiocarbonyl)-β-D-xylo- and ribo-pentofuranosyl] thymine (11) (0.53 g, 82%) as a syrup. ^1H NMR ($CDCl_3$, 90 MHz): δ 0.86 (s, 18H, t-Bu), 1.90 (s, 3H, 5-CH_3), 4.00-4.33 (m, 2H, H-5'), 4.50 (m, 1H, H-4' minor epimer), 4.65 (m, 1H, H-4' major epimer), 6.30 (d, 1H $J_{1',2'}$ 6.5 Hz, H-2'), 6.52 (d, 1H, H-1'), 6.96-7.66 (m, 5H, C_6H_5), 9.00 (bs, 1H, 3-NH major), 9.20 (bs, 1H, 3-NH minor).

Syrupy compound 11 obtained before, was suspended in toluene (20 mL) and transferred to a three neck flask. α,α'-Azobisisobutironitrile (0.03 g, 0.2 mmol) was added, oxigen free N_2 was bubled through the suspension for 15 min, and then tributyltin hydride (0.4 mL, 1.5 mmol) was added. The flask was heated in an oil bath at 70°C for 4 h, while the N_2 bubbling was maintained. The heating was removed until the reaction mixture reached the room temp. Then, it was washed with water (1 x 20 mL), dried over anhydrous sodium sulfate, filtered, and the filtrate evaporated under reduced pressure. The residue was purified through a silica gel (20 g) column chromatography using hexane/ethyl acetate (4:1) as the eluent to afford 14 (0.29 g, 60% from 9) as a white foam. $[\alpha]_D$ + 32° (c 0.5, $CHCl_3$); ν_{max} (Nujol) 2225 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 90 MHz): δ 0.80, 0.93 (2s, 18H, t-Bu), 1.90 (s, 3H, 5-CH_3), 3.23 (dd, 1H, $J_{2',3'}$ 7.3, $J_{3',4'}$ 8 Hz, H-3'), 3.91 (m, 2H, H-5'), 4.41 (m, 1H, H-4'), 4.52 (dd, 1H, $J_{1',2'}$ 6 Hz, H-2'), 5.90 (d, 1H, H-1'), 7.35 (s, 1H, H-6), 9.30 (bs, 1H, 3-NH). Anal. Calcd. for $C_{23}H_{41}N_3O_5Si$: C, 55.72; H, 8.33; N, 8.47. Found: C, 55.59; H, 8.33; N, 8.12.

1- (3'-C-Cyano-3'-deoxy-β-D-xylo-pentofuranosyl)thymine (15). A mixture of 14 (0.49 g, 1 mmol), tetrahydrofuran (15 mL), and tetrabutylammonium fluoride trihydrate (0.63 g, 2 mmol) was stirred at room temperature for 1 h. The reaction mixture was filtered through a short column of silica gel (10 g) using tetrahydrofuran as the eluent. The solvent was evaporated under reduced pressure and the brown residue was purified by column chromatography using ethyl acetate/methanol (50:1) as the eluent to afford 15 (0.16 g, 61%); m.p. 194-5°C (dec.) (from ethyl acetate) ν_{max} (BrK) 2230 cm^{-1} (CN); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.79 (d, 3H, J 1.0 Hz, 5-CH_3), 3.62 (m, 1H, H-3', after addition of D_2O, dd, $J_{2',3'}$ 8.1, $J_{3',4'}$ 8.4 Hz), 3.65 (m, 2H, H-5'), 4.34 (m, 1H, H-4', after addition of D_2O, dt), 4.50 (m, 1H, H-2', after addition of D_2O, dd, $J_{1',2'}$ 6.5 Hz), 5.45 (t, 1H, $J_{5',OH}$ 4.3 Hz, 5'-OH), 5.76 (d, 1H, H-1'), 6.26 (d, 1H, $J_{2',OH}$ 5.8 Hz, 2'-OH), 7.73 (q, 1H, H-6), 11.40 (bs, 1H, 3-NH). Anal. Calcd. for $C_{11}H_{13}N_3O_5$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.38; H, 5.07; N, 15.58.

1- [5'-O-(t-Butyldimethylsilyl)-3'-C-cyano-3'-deoxy-β-D-xylo-pentofuranosyl] thymine (16). A cold (ice bath), stirred solution of 15 (0.27 g, 1 mmol) in pyridine (8 mL) was treated with t-butyldimethylsilyl chloride (0.17 g, 1.1 mmol). The ice bath was removed and the temperature slowly rised to 18 C. The stirring was maintained for 24 h. The solvents were evaporated under reduced pressure and the residue, dissolved in ethyl acetate (25 mL) was washed with 1N HCl (25

mL), and with water (2 x 25 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crystallized from chloroform/hexane to afford 16 (0.35 g, 93%) as a white solid; m.p. 189-90°C; ν_{max} (KBr) 2225 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 90 MHz): δ 0.93 (s, 9H, t-Bu), 1.85 (s, 3H, 5-CH_3), 3.42 (dd, 1H, $J_{2',3'}$ 6, $J_{3',4'}$ 7.2 Hz), 3.96 (m, 2H, H-5'), 4.50 (m, 1H, H-4'), 4.67 (m, 1H, H-2', collapses to dd on D_2O shake, $J_{1',2'}$ 3.5 Hz), 5.43 (d, 1H, 2'-OH), 5.82 (d, 1H, H-1'), 7.40 (s, 1H, H-6). Anal. Calcd. for $C_{17}H_{27}N_3O_5Si$: C, 53.52; H, 7.13; N, 11.01. Found: C, 53.41; H, 7.01; N, 11.00.

1- [2',5'-Di-O-(t-butyldimethylsilyl)-3'-C-cyano-3'-deoxy- β-D-ribo-pentofuranosyl] thymine (18). A 1N solution of NaOH in methanol was added dropwise to a solution of 14 (0.4 g, 0.8 mmol) in methanol (15 mL) until the pH of the resulting solution was 9. The reaction mixture was heated to reflux for 5 h, neutralized with a 1 N solution of HCl in methanol and evaporated to dryness. The residue was purified by column chromatography with hexane/ethyl acetate (4:1) as the eluant to yield 18 (0.30 g, 76%) as a syrup; (Rf 0.45); ν_{max} (Film) 2230 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 300 MHz): δ 0.93 (s, 18H, t-Bu), 1.89 (d, 3H, J 0.8 Hz, 5-CH_3), 3.19 (dd, 1H, $J_{2',3'}$ 4.4, $J_{3',4'}$ 10.0 Hz, H-3'), 3.88 (dd, 1H, $J_{4',5'a}$ 2.0; $J_{5'a,5'b}$ 12.2 Hz, H-5'a), 4.27 (dd, 1H, $J_{4',5'b}$ 1.5 Hz, H-5'b), 4.52 (ddd, 1H, H-4'), 4.55 (dd, 1H, $J_{1',2'}$ 0.8 Hz, H-2'), 5.66 (d, 1H, H-1'), 7.47 (q, 1H, H-6), 8.49 (bs, 1H, 3-NH). Anal. Calcd. for $C_{23}H_{41}N_3O_5Si$: C, 55.72; H, 8.33; N, 8.47. Found: C, 55.55; H, 8.08; N, 8.31.

From this reaction a minor, slower running syrup was also isolated which was identified as 1-[2'-O-(t-butyldimethylsilyl)-3'-C-cyano-3'-deoxy-β-D-ribo-pentofuranosyl]thymine (19). (0.024 g, 6%.); (Rf 0.35, hexane/ethyl acetate, (4:1)); ν_{max} (Film) 2230 cm^{-1} (CN), 3420 cm^{-1} (OH); ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (s, 9H, t-Bu), 1.86 (d, 3H, J 1.0 Hz, 5-CH_3), 2.92 (bs, 1H, 5'-OH), 3.49 (dd, 1H, $J_{2',3'}$ 4.9, $J_{3',4'}$ 9.5 Hz, H-3'), 3.84 (dd, 1H, $J_{4',5'a}$ 1.8, $J_{5'a,5'b}$ 12.6 Hz, H-5'a), 4.14 (dd, 1H, $J_{4',5'b}$ 1.8 Hz, H-5'b), 4.52 (dt, 1H, H-4'), 4.66 (dd, 1H, $J_{1',2'}$ 1.5 Hz, H-2'), 5.50 (d, 1H, H-1'), 7.55 (q, 1H, H-6), 9.35 (bs, 1H, 3-NH). Anal. Calcd. for $C_{17}H_{27}N_3O_5Si$: C, 53.52; H, 7.13; N, 11.01. Found: C, 53.30; H, 7.19; N, 10.89.

1- [5'-O-(t-Butyldimethylsilyl)-3'-C-cyano-2',3'-dideoxy- β-D-threo-pentofuranosyl] thymine (23). A mixture of 16 (0.38 g, 1 mmol), methylene chloride (15 mL), pyridine (0.3 mL, 4 mmol) and phenyloxythiocarbonyl chloride (0.2 mL, 1.1 mol) was stirred at room temp for 2 h. The solvents were evaporated at reduced pressure and the residue was suspended in toluene (20 mL) and transferred to a three necked flask. α,α'-Azobisisobutironitrile (0.03 g, 0.2 mmol) was added, oxigen free N_2 was bubbled through the suspension for 15 min, and then tributyltin hydride (0.4 mL, 1.5 mmol) was added. The reaction flask was heated in an oil bath at 70 C for 3 h, while the N_2 bubbling was maintained. The reaction was allowed to reach room temperature, washed with water (20 mL), dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated to dryness and the residue was purified by silica gel (10 g) column chromatography using hexane/ethyl acetate (3:1) as the eluent to afford 23 (0.21 g, 60%) as a white foam; ν_{max} (Nujol) 2225 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 90 MHz): δ 0.90 (s, 9H, t-Bu), 1.93 (s, 3H, 5-CH_3), 2.34 [ddd, 1H, $J_{1',2'(\beta)}$ 6.8, $J_{2'(\alpha),2'(\beta)}$ 13.9, $J_{2'(\beta),3'}$ 7.2 Hz, H-2'(β)], 2.75 [ddd, 1H, $J_{1',2'(\alpha)}$ 6.8, $J_{2'(\alpha),3'}$ 7.2 Hz, H-2'(α), 3.43 (q, 1H, $J_{3',4'}$ 7.4 Hz, H-3'), 4.01 (d, 2J, $J_{4',5'}$ 3.7 Hz, H-5'), 4.23 (dt, 1H, H-4'), 6.18 (t, 1H, H-1'), 7.46 (s, 1H, H-6), 9.55 (bs, 1H, 3-NH). Anal. Calcd. for $C_{17}H_{27}N_3O_4Si$: C, 55.86; H, 7.44; N, 11.50. Found: C, 55.65; H, 7.40; N, 11.69.

1-(3'-C-Cyano-2',3'-dideoxy-β-D-threo-pentofuranosyl)thymine (24). Compound 23 (0.27 g, 0.75 mmol) was stirred at room temp with a 0.1 N solution of HCl in methanol for 30 min. The reaction mixture was neutralized with a 1 N solution of NaOH in methanol and the solvent was evaporated to dryness. The residue was crystallized from ethyl acetate to afford 24 (0.17 g, 94%); m.p. 201-2 C; $[\alpha]_D$ + 26° (c 0.5 DMSO); ν_{max} (KBr) 2230 cm^{-1} (CN); λ_{max} (MeOH) 262 nm (ε, 8600); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.78 (d, 3H, J 1.1 Hz, 5-CH_3), 2.30 [ddd, 1H, $J_{1',2'(\beta)}$ 6.8, $J_{2'(\alpha),2'(\beta)}$ 13.1, $J_{2'(\beta),3'}$ 7.1 Hz, H-2'(β)], 2.65 [ddd, 1H, $J_{1',2'(\alpha)}$ 6.6, $J_{2'(\alpha),3'}$ 8.1 Hz, H-2'(α)], 3.74 (m, 3H, H-3', H-5'), 4.16 (dt, 1H, $J_{3',4'}$ 7.1, $J_{4',5'}$ 4.4 Hz, H-4'), 5.30 (t, 1H, $J_{5',OH}$ 4.6 Hz, 5'-OH), 6.07 (t, 1H, H-1'), 7.65 (q, 1H, H-6); ^{13}C NMR [$(CD_3)_2SO$, 75 MHz]: δ 12.37 (5-CH_3), 29.45 (C-3'), 36.64 (C-2'), 61.49 (C-5'), 78.38, 83.35 (C-1', C-4'), 109.76 (C-5), 119.400 (CN), 135.65

a(C-6), 150.46 (C-2), 163.77 (C-4). Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.21; N, 16.72. Found: C, 52.43; H, 5.38; N, 16.88.

5'-O-(t-Butyldimethylsilyl)-3'-C-cyano-3'-deoxythymidine (25). Compound 23 (0.73 g, 2 mmol) was epimerized following method A, described before for 18, to afford 25 (0.55 g, 76%) as a syrup. ν_{max} (Film) 2230 cm^{-1} (CN); ^1H NMR (CDCl3, 90 MHz): δ 0.90 (s, 9H, t-Bu), 1.89 (s, 3H, 5-CH_3), 2.41 [ddd, 1H, $J_{1',2'(\beta)}$ 5, $J_{2'(\alpha),\ 2'(\beta)}$ 13.6, $J_{2'(\beta),3'}$ 8.6 Hz, H-2'(β)], 2.67 [ddd, 1H, $J_{1',2'(\alpha)}$ 6.5, $J_{2'(\alpha),\ 3'}$ 8.6 Hz, H-2'(α)], 3.36 (q, 1H, $J_{3',4'}$ 8.2 Hz, H-3'), 3.94 (m, 2H, H-5'), 4.25 (dt, 1H, H-4'); 6.16 (dd, 1H, H-1'), 7.33 (s, 1H, H-6), 9.62 (bs, 1H, 3-NH). Anal. Calcd. for $C_{17}H_{27}N_3O_4Si$: C, 55.86; H, 7.44; N, 11.50. Found: C, 55.85; H, 7.21; N, 11.66.

3'-C-Cyano-3'-deoxythymidine (26). Compound 25 (0.36 g, 1 mmol) was treated with 0.1 N HCl in methanol and worked up, as described before for 24, to afford 26 (0.23 g, 91%); m.p. 132-3 C (from ethyl acetate/hexane) Lit.[7] m.p. 133-4°C]; $[\alpha]_D$ + 34° (c 0.5 MeOH) [Lit.[7] $[\alpha]_D$ + 32.4 (c 0.25 MeOH)]; ν_{max} (Nujol) 2240 cm^{-1} (CN); ^1H NMR [$(CD_3)_2SO$, 300 MHz]: δ 1.78 (d, 3H, J 0.8 Hz, 5-CH_3), 2.47 [ddd, 1H, $J_{1',2'(\beta)}$ 4.2, $J_{2'(\alpha),\ 2'(\beta)}$ 13.5, $J_{2'(\beta),3'}$ 9.1 Hz, H-2'(β)], 2.66 [ddd, 1H, $J_{1',2'(\alpha)}$ 7.5, $J_{2'(\alpha),\ 3'}$ 9.2 Hz, H-2'(α)], 3.50 (q, 1H, $J_{3',4'}$ 8.8 Hz, H-3'), 3.64 (m, 1H, $J_{5'a,5'b}$ 13.2 Hz, H-5'a), 3.70 (m, 1H, H-5'b), 4.13 (dt, 1H, H-4'), 5.32 (bs, 1H, 5'-OH), 6.15 (dd, 1H, H-1'), 7.63 (q, 1H, H-6), 10.86 (bs, 1H, 3-NH); ^{13}C NMR [$(CD_3)_2SO$, 50 MHz]: δ 12.12 (5-CH_3), 27.68 (C-3'), 34.87 (C-2'), 60.15 (C-5'), 82.37, 83.95 (C-1', C-4'), 109.22 (C-5), 119.59 (CN), 136.23 (C-6), 150.30 (C-2), 163.71 (C-4). Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.21; N, 16.72. Found: C, 52.51; H, 5.11; N, 16.58.

5-O-(t-Butyldimethylsilyl)-3-C-cyano-1,2-O-isopropylidene-3-O-(phenyloxythiocarbonyl)-α-D-ribofuranose (28). A solution of compound 27[21] (0.98 g, 3 mmol) in acetonitrile (30 mL) was treated with 4-dimethylaminopyridine (0.75 g, 6.1 mmol) and phenyloxythiocarbonyl chloride (0.6 mL, 3.3 mmol). The mixture was stirred at room temp for 3 h and evaporated to dryness. The residue was chromatographed on tlc plates using hexane/ethyl acetate (6:1) as the eluent to yield 28 (1.24 g, 89%) as a syrup; $[\alpha]_D$+ 67° (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 90 MHz): δ 0.76 (s, 9H, t-Bu), 1.20, 1.47 (2s, 6H, isopropylidene), 3.96 (m, 2H, H-5), 4.31 (m, 1H, H-4), 5.30 (d, 1H, $J_{1,2}$ 4 Hz, H-2), 5.86 (d, 1H, H-1), 6.93-7.40 (m, 5H, C_6H_5). Anal. Calcd. for $C_{22}H_{31}NO_6SSi$: C, 56.75; H, 6.71; N, 3.01. Found: C, 56.55; H, 6.57; N, 2.80.

5-O-(t-Butyldimethylsilyl)-3-C-cyano-1,2-O-isopropylidene-3-O-(phenyloxythiocarbonyl)-α-D-xylofuranose (32). A solution of compound 31[21] (0.66 g, 2 mmol) in acetonitrile (25 mL) was reacted with 4-dimethylaminopyridine (0.50 g, 4.1 mmol) and phenyloxythiocarbonyl chloride (0.4 mL, 2.2 mmol) and was worked up as described before for 28, to afford 32 (0.83 g, 91%) as a syrup which spontaneously crystallized; m.p. 136-7°C; $[\alpha]_D$ + 49° (c 0.5 $CHCl_3$); ^1H NMR ($CDCl_3$, 90 MHz): δ 0.75 (s, 9H, t-Bu), 1.21, 1.47 (2s, 6H, isopropylidene), 3.90 (d, 2H, $J_{4,5}$ 6.5 Hz, H-5), 4.53 (t, 1H, H-4), 5.12 (d, 1H, $J_{1,2}$ 4 Hz, H-2), 5.86 (d, 1H, H-1), 6.90-7.33 (m, 5H, C_6H_5). Anal. Calcd. for $C_{22}H_{31}NO_6SSi$: C, 56.75; H, 6.71; N, 3.01. Found: C, 56.57; H, 6.81; N, 2.81.

5-O-Acetyl-3-C-cyano-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (30). From 28. A three-necked flask fitted with magnetic stirrer, reflux condenser and nitrogen inlet was charged with compound 28 (0.46 g, 1 mmol), toluene (20 mL), and α,α'-azobisisobutironitrile (0.032 g, 0.2 mmol). Oxigen free nitrogen was bubbled through the mixture for 15 min and tributyltin hydride (0.4 mL, 1.5 mmol) was added. The flask was heated in an oil bath at 70°C for 3 h while the nitrogen flow was maintained. On cooling to room temperature, the reaction mixture was washed with water (1 x 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was chromatographed (column) using hexane/ethyl acetate (4:1) to give 0.27 g of a syrup which was a (10:1) mixture of two compounds with identical chromatographic mobilities in several solvent systems. The minor compound was 5-O-(t-butyldimethylsilyl)-1,2-O-isopropyline-α-D-erythro-pentofuranos-3-ulose which may be formed from cyanohydrin 27. The major compound was 5-O-(t-butyldimethylsilyl)-3-C-cyano-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (29). ^1H NMR ($CDCl_3$, 90 MHz): δ 0.83 (s, 9H, t-Bu), 1.30, 1.51 (2s, 6H, isopropylidene), 3.03 (dd, 1H, $J_{2,3}$ 5, $J_{3,4}$ 9.5 Hz, H-3), 3.80 (m, 2H, H-5), 4.25 (m, 1H, H-4), 4.75 (dd, 1H, $J_{1,2}$ 4 Hz, H-2), 5.80 (d, 1H, H-1).

This compound was fully characterised as its 5-*O*-acetyl derivative **30** as indicated as follows.

The above (10:1) mixture (0.27 g) was treated with a 0.1 N solution of HCl in methanol (10 mL) with stirring for 30 min. The reaction was neutralised with 1 N NaOH in methanol and the solvent was evaporated to dryness. The residue was acetylated with acetic ahydride (0.5 mL) and pyridine (8 mL) at room temperature for 6 h. The solvents were evaporated under reduced pressure and the residue was purified by column chromatography with hexane/ethyl acetate (2:1) to afford **30** (0.165 g, 66%); m.p. 101-2 C° (from hexane); $[\alpha]_D$ + 121° (*c* 0.5, chloroform); ν_{max} (KBr) 2235 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 90 MHz): δ 1.37, 1.60 (2s, 6H, isopropylidene), 2.10 (s, 3H, OAc), 2.90 (dd, 1H, $J_{2,3}$ 5, $J_{3,4}$ 9 Hz, H-3), 4.15-4.60 (m, 3H, H-4, H-5), 4.83 (dd, 1H, $J_{1,2}$ 4 Hz, H-2), 5.90 (d, 1H, H-1). Anal. Calcd. for $C_{11}H_{15}NO_5$: C, 54.76; H, 6.26; N, 5.80. Found: C, 54.96; H, 6.30; N, 6.10.

Nuclear Overhauser Effect Experiments. ^1H NMR steady-state NOE diference spectroscopy experiments were carried out on compounds **12**, **24** and **26** with a Brucker AM 200 spectrometer operating in the pulse mode. The standard Brucker microprogram library was used to perform sequential multiplet line irradiation.[22] Each irradiation multiplet frecuency was cycled 20 times before adquisition. A total irradiation time of 2s and an adquisition time of 2s was used. Solutions ($CDCl_3$+Me_4Si) were measured at 30 C and a 90 read pulse was used in all cases. The decoupling power was adjusted in order to obtain maximum saturation (80-90%) compatible with minimum frecuency spillover to neighbouring multiplets. F.I.D. were weighted with a 2 Hz exponential line-broadening factor, substracted and fourier transformed. NOE values were calculated from integrals of the difference and control irradiation spectra.

ACKNOWLEDGEMENTS. The authors thank Mrs. María-Jesús Moreno Guerrero for technical assistance and The Consejo Superior de Investigaciones Científicas (C.S.I.C.) and the C.A.I.C.Y.T. for financial support.

REFERENCES

1. De Clercq, E. TIPS, 1987, 8, 339.
2. Mitsuya, H.; Broder, S. Nature, 1987, 325, 773.
3. De Clercq, E. J. Med. Chem., 1986, 29, 1561.
4. Fischl, M.A.; Richman, D.D.; Grieco, M.H.; Gottlieb, M.S,; Wolberding, P.A.; Laskin, O.L.; Leedom, J.M.; Groopman, J.E.; Mildvan, D.; Schooley, R.T.; Jackson, G.G.; Durack, D.T.; Phil, D.; King, D. New Engl. J. Med., 1987, 317, 185.
5. Yarchoan, R.; Berg, G.; Brouwers, P.; Fischl, M.A.; Spitzer, A.R.; Wichman, A.; Grafman, J.; Thomas, R.V.; Safai, B.; Brunetti, A.; Perno, C.F.; Schmidt, P.J.; Larson, S.M.; Myers, C.E.; Broder, S. Lancet, i, 1987, 132.
6. De las Heras, F.G.; San Felix, A.; Calvo-Mateo, A.; Fernández-Resa, P. Tetrahedron, 1985, 41, 3867.
7. Fleet, G.W.J.; Son, J.C.; Derome, A.E. Tetrahedron, 1988, 44, 625.
8. Ashwell, M.; Jones, A.S.; Walker, R.T. Nucleic Acids Res., 1987, 15, 2157.
9. Calvo-Mateo, A.; Camarasa, M.J.; Díaz-Ortíz, A.; De las Heras, F.G. Tetradedron Lett., 1988, 29, 941.
10. Nishimura, T.; Shimizu, B.; Iwai, I. Chem. Pharm. Bull. (Tokyo), 1964, 12, 1471.
11. Hansske, F.; Madej, D.; Robins, M.J. Trtrahedron, 1984, 40, 125.
12. Hartwig, W. Tetrahedron, 1983, 39, 2609.
13. Robins, M.J.; Wilson, J.S. J. Am. Chem. Soc., 1981, 103, 932.
14. Jain, T.C.; Jenkins, I.D.; Russell, A.F.; Verheyden, J.P.H.; Moffatt, J.G. J. Org. Chem. 1974, 39, 30.
15. Robins, M.J.; Jones, R.A.; Mengel, R. Can. J. Chem., 1977, 55, 1251.
16. Sasaki, T.; Minamoto, K.; Hattori, K. J. Org. Chem., 1973, 38, 1283.
17. Sasaki, T.; Minamoto, K.; Suzuki, H. J. Org. Chem., 1973, 38,598.
18. Furukawa, Y.; Yoshioka, Y.; Imai, K.; Honjo, M. Chem. Pharm. Bull., 1970, 18, 554.
19. Bernstein, M.A.; Morton, H.E.; Guindon, Y. J. Chem. Soc. Perkin II, 1986, 1155.
20. Yoshimura, J. Adv. Carbohydr. Chem. Biochem., 1984, 42, 82.
21. Calvo-Mateo, A.; Camarasa, M.J.; Díaz-Ortíz, A.; De las Heras, F.G. J. Chem. Soc. Chem. Commun., in Press.
22. Kinns, M.; Sanders, J.R.M. J. Magn. Reson. 1984, 56, 518.