SYNTHESIS, CONFORMATION ANALYSIS AND BIOLOGICAL EVALUATION OF 2-(2,3-DIDEOXY-β-D-RIBOFURANOSYL)PYRIDINE-4-CARBOXAMIDE

Pieter E. Joos[a*], Eddy L. Esmans[a], Frank C. Alderweireldt[a], André De Bruyn[b] Jan Balzarini[c], and Erik De Clercq[c]

[a]University of Antwerp (RUCA), Department of Chemistry, *Groenenborgerlaan* 171, B-2020-Antwerp, Belgium

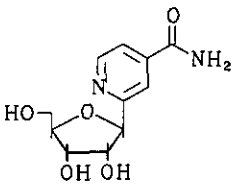
[b]University of Ghent (RUG), Laboratory for Organic Chemistry, Krijgslaan 281, B-9000-Ghent, Belgium

[c]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000-Leuven, Belgium

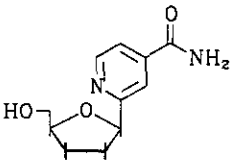
Abstract - 2-(2,3-Dideoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (2) was synthesized starting from the protected sugar analogue (3). Coupling of this compound with 2-lithio-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (4), subsequent mesylation, reduction, deprotection and ammonolysis gave (2) as an anomeric mixture. α/β-Separation was done by semi preparative hplc on a Lichrosorb 10 RP 8 column. In addition 2D-NOESY nmr spectroscopy was used for the assignment of the α/β-anomers of (2). High resolution ^{1}H nmr allowed us to do a conformation analysis of compound (β-2). Finally the β-anomer of (2) was evaluated for its biological activity.

INTRODUCTION

The discovery that thiazofurin has cytostatic properties as a result of the enzyme inosine monophosphate-dehydrogenase,[1] and the fact that a S-atom is isoster with a double bond and thus can be replaced by it, the synthesis of pyridine-C-nucleosides was investigated. Within the framework of a structure activity relation (SAR) study several of these compounds have been synthesized, of which some showed mild cytostatic activity.[2,3] Furthermore 2',3'-dideoxynucleosides of the pyrimidine and purine type have attracted interest as potential anti-HIV-agents. The 2',3'-dideoxynucleosides, 3'-azido-2',3'-dideoxythymidine (AZT),[4,5] 2',3'-dideoxycytidine (ddC)[6,7] and 2',3'-dideoxyinosine (ddI)[6,8] have been formally licensed for clinical use in HIV-infected patients. These compounds are metabolized in vivo to the corresponding 5'-triphosphates. The latter compounds inhibit the reverse transcriptase, an enzyme which is essential for the synthesis of DNA from viral RNA. In view of the above and within the context of a SAR study of the biological activity of pyridine-C-nucleosides we wish to report on the synthesis and the conformational analysis of 2-(2,3-dideoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (2). (Figure 1)



(1)



(2)

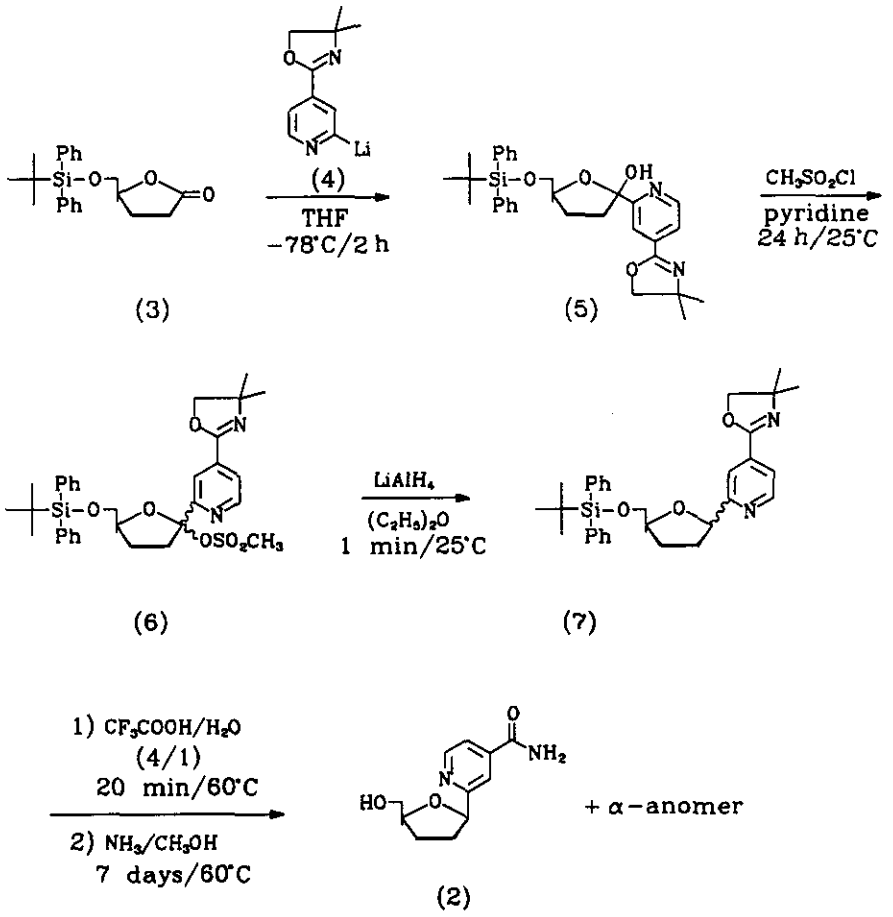
Figure 1: Structures of 2-β-D-ribofuranosylpyridine-4-carboxamide 1[2] and its 2',3'-dideoxyanalogue (2)

RESULTS AND DISCUSSION

2',3'-Dideoxy derivatives of naturally occurring nucleosides were first synthesized by Robins[9,10] and by Horwitz et al.[11,12] In general two methods were developed. One of them[13] used a protected sugar compound appropriately derivatised at C(1'), which is substituted at the anomeric carbon atom by a selected pyrimidine or purine base. This method has evolved as a suitable pathway for the synthesis of N-nucleosides. However it results in low yields of C-nucleosides of the ribo-[14] and 2'-deoxyribo-type[15].

In a second method the 2'- and/or 3'-hydroxylfunction(s) are reduced by either the Barton-McCombie method[15] or the Corey-Winter procedure.[16] However these methods start from the corresponding ribo- or 2'-deoxyribonucleosides and synthesizing this compound consequently lengthen the synthesis.

For the synthesis of the title compound we selected an approach based upon the use of 5-O-tert-butyldiphenylsilyl-2,3-dideoxy-D-ribono-1,4-lactone (3) and a lithiopyridine (Scheme 1). Such a method in which a lactone is chosen, has already been used for the synthesis of D-ribofuranosylnucleosides.[16,17]



Scheme 1: Synthesis of 2-(2,3-dideoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (β-2)

Commercially available (S)-4-hydroxymethylbutyro-1,4-lactone was protected at the C(5')-OH-function with (tert-butyl)diphenylsilyl chloride. This protecting group is, as already reported by us[18], stable under

the lithiation conditions generally used. It can be removed by TBAF or CF_3COOH/H_2O (4/1).

2-Lithio-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (4) was obtained by a metal-halogen exchange reaction between butyllithium and 2-bromo-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine in THF at -78 °C.[2,20] To this solution, a solution of 5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-D-ribonolactone (3) in THF was added and the compounds were allowed to react for another 2 h at -78 °C. After extraction with AcOEt and purification by Centrifugal Circular Thin Layer Chromatography (CCTLC, Chromatotron^R eluant : AcOEt), the lactole (5) was isolated in 55% yield.

From the nmr data (Table 1) we concluded that in this reaction only one isomer of compound (5) was formed. This was not a complete surprise since we could expect that the extremely bulky substituent on C(5') (the tert-butyldiphenylsilyloxy group) would hinder the approach of the lithiopyridine from the si-face of the ring. This results in a β-OH-configuration for compound (5). The same phenomenon was also observed by Watanabe and co-workers[18] during the addition of 2-lithio-6-bromopyridine to 5-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-D-ribonolactone and by Piccirilli et al.[19] during additions of various lithiopyridines to 5-O-[(tert-butyl)diphenylsilyl]-2,3-O-isopropylidene-D-ribonolactone. The latter authors mention a 7/1 ratio in favor of the β-OH-isomer, while the Watanabe group did not observe any α-OH isomer at all.

Treatment of (5) with methanesulfonyl chloride in dry pyridine gave the mesylate (6) in an 80% yield. During this reaction isomerisation was observed (ratio 75/25 as determined by integration of the H-C(3)-proton in the ^1H nmr spectrum of compound (6)). Attempts to separate the anomeric mixture of the mesylates (α-$MeSO_2$-6) and (β-$MeSO_2$-6) failed. The corresponding nmr data of (α-$MeSO_2$-6) and (β-$MeSO_2$-6) are summarized in Table 1.

For the following reduction step we suggest a S_N2-mechanism. This reaction gave the same ratio of α/β-anomers. Since the absolute configuration could be determined in a following step (see NOESY-spectra), we consequently could assign the absolute configuration of the compounds (α-$MeSO_2$-6) and (β-$MeSO_2$-6), although we admit that this assignment is tentative and fully depends on the assumption of a S_N2-mechanism.

Table 1: 500 MHz ^1H nmr data of compounds (5), (α-$MeSO_2$-6)[a] and (β-$MeSO_2$-6)[a]

Compound	(5) ($CDCl_3$/TMS)	(α-$MeSO_2$-6) ($CDCl_3$/TMS)	(β-$MeSO_2$-6) ($CDCl_3$/TMS)
H_a-C(2')	2.18 (m)	2.25-2.33 (m)	2.48 (m)
H_b-C(2')	2.02 (m)	2.50 (m)	1.85-2.20 (m)
H_a-C(3')	1.75-1.85 (m))	1.85-2.20 (m)	
H_b-C(3')			
H-C(4')	3.79 (m)	4.50 (m)	4.43 (m)
H_a-C(5')	3.67 (dd)	3.80 (dd)	3.79 (dd)
H_b-C(5')	3.56 (dd)	3.89 (dd)	3.81 (dd)
H-C(3)	8.43 (br s)	8.09 (dd)	8.19 (dd)
H-C(5)	7.92 (dd)	7.95 (dd)	[b])
H-C(6)	8.69 (dd)	8.71 (dd)	8.72 (dd)
CH_3 (tert-butyl)	1.04 (s)	0.94 (s)	0.94 (s)
H_o	7.65-7.75 (m)	7.60-7.68 (m)	7.60-7.68 (m)
H_m/H_p	7.30-7.40 (m)	7.25-7.38 (m)	7.25-7.38 (m)
CH_3 ($MeSO_2$)	-	2.99 (s)	3.07 (s)
CH_2 (oxazole)	4.13 (s)	4.15 (s)	4.14 (s)
CH_3 (oxazole)	1.37 (s)	1.32 (s)	1.28 (s)
J(2'a,2'b)	[b])	-11.9 Hz	-9.5 Hz
J(2'a,3'a)	[b])	[b])	3.4 Hz
J(2'a,3'b)	[b])	[b])	8.1 Hz
J(2'b,3'a)	[b])	1.2 Hz	[b])
J(2'b,3'b)	[b])	5.2 Hz	[b])
J(3'a,3'b)	[b])	[b])	[b])
J(3'a,4')	[b])	8.2 Hz	8.3 Hz
J(3'b,4')	[b])	5.4 Hz	5.2 Hz
J(4',5'a)	4.0 Hz	6.0 Hz	4.1 Hz
J(4',5'b)	6.7 Hz	6.5 Hz	3.9 Hz
J(5'a,5'b)	-10.1 Hz	-10.8 Hz	-10.5 Hz
J(3,5)	1.7 Hz	1.7 Hz	1.7 Hz
J(3,6)	0.8 Hz	0.9 Hz	0.9 Hz

J(5,6)	5.0 Hz	4.9 Hz	5.0 Hz

a) $MeSO_2{=}OSO_2CH_3$
b) Value could not be determined due to overlap.

Subsequent reduction of the mesylates (α/β-$MeSO_2$-6) gave the fully protected nucleosides (α/β-7) in reasonable yields (51%). Removal of the protecting groups by CF_3COOH/H_2O (4/1) at 60 °C and subsequent ammonolysis resulted in the formation of the α/β-mixture of (2). Purification of the α/β-mixture of (2) was done by CCTLC (Chromatotron^R; eluant : CH_2Cl_2/CH_3OH 90/10 and 85/15 respectively). The α/β-mixture of (2) was separated into the two anomers by the aid of semi preparative hplc (Lichrosorb 10 RP 8, 25 cm x 22.5 mm ID, flow=8 ml/min, H_2O/CH_3OH 85/15, detection at λ=270 nm, $t_R(\alpha)$=47.99 min, $t_R(\beta)$=41.81 min) (yield : 65% from (7)). An α/β-ratio of 26/74 was obtained from integration of the chromatographic peaks. ^{1}H Nmr data of (α-2) and (β-2) are reproduced in Table 2.

Table 2: 500 MHz ^{1}H nmr data of compounds (α-2) and (β-2).

Compound	(α-2) (CD_3OD/ DMSO-d_6 (1/1))	(β-2) (CD_3OD/ DMSO-d_6 (1/1))
H-C(3)	7.87 br s	7.83 br s
H-C(5)	7.60 dd	7.59 dd
H-C(6)	8.56 d	8.56 d
H-C(1')	4.94 t	5.02 t
H_α-C(2')	2.32 m	2.36 m
H_β-C(2')	1.84 m	1.87 m
H_α-C(3')	1.96 m	1.95 m
H_β-C(3')	1.76 m	1.76 m
H-C(4')	4.09 m	4.25 m

H_a-C(5')	3.58 dd	3.50 dd
H_b-C(5')	3.52 dd	3.47 dd
J(3,5)	1.7 Hz	1.7 Hz
J(5,6)	5.0 Hz	5.1 Hz
J(1',2'α)	7.3 Hz	7.1 Hz
J(1',2'β)	7.1 Hz	7.4 Hz
J(2'α,2'β)	-11.9 Hz	-11.7 Hz
J(2'α,3'α)	7.2 Hz	4.3 Hz
J(2'α,3'β)	5.9 Hz	7.5 Hz
J(2'β,3'α)	7.4 Hz	7.4 Hz
J(2'β,3'β)	8.5 Hz	7.8 Hz
J(3'α,3'β)	-12.1 Hz	-11.7 Hz
J(3'α,4')	6.8 Hz	6.7 Hz
J(3'β,4')	6.8 Hz	7.1 Hz
J(4',5'a)	4.7 Hz	4.6 Hz
J(4',5'b)	4.8 Hz	5.1 Hz
J(5',5'b)	-11.6 Hz	-11.4 Hz

Assignment of the anomeric configuration was done with the aid of 2D-NOESY spectrometry. In the presumed α-isomer, a cross-peak between H-C(1') and H-C(5'a) and H-C(5'b) was observed (Figure 2), while in the spectrum of the presumed β-isomer (Figure 3), no signal was seen.

Moreover, in the NOESY spectrum of the β-isomer (Figure 3), there was a cross-peak between the H-C(5'a)-signal and the protons of the heterocyclic base and between the base and the H-C(2'β), while this cross-peak had totally disappeared in the NOESY spectrum of the α-anomer. In the NOESY spectrum of the latter a cross-peak of H-C(6) and the H-C(2'α)-signal was seen, while this phenomenon did not occur in the spectrum of the β-anomer. Other cross-peaks are probably due to diffusion effects, which are *dependent on mixing times.*

Numbering of the protons is as depicted in Figure 4.

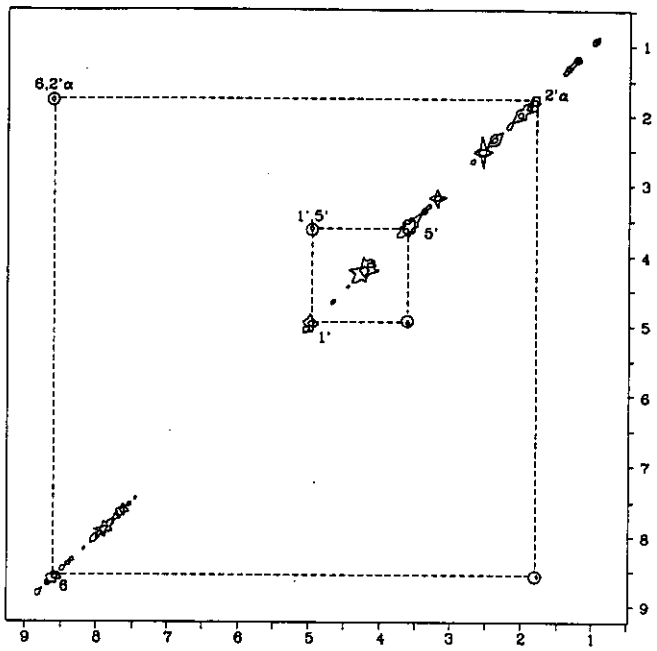


Figure 2: NOESY-spectrum of compound (α-2)

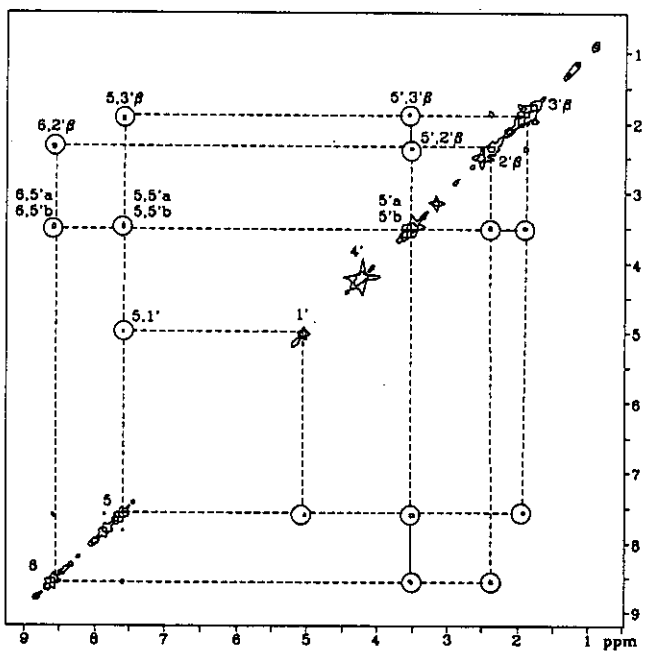


Figure 3: NOESY-spectrum of compound (β-2)

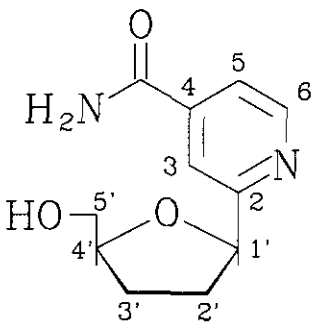


Figure 4: Numbering of the atoms of compound (2)

BIOLOGICAL STUDIES

The antiviral and cytostatic properties of compounds (β-2) were evaluated in a variety of assay systems (Table 3). The compound (β-2) did not exhibit any appreciable antiviral or cytostatic effect in any of the assay systems.

Table 3: Biological evaluation of compound (β-2)

ANTIVIRAL ACTIVITY	Minimum inhibitory concentration (μg/ml) [a])
Assay system	(β-2)
Human immunodeficiency virus type 1/MT-4	>200
Human immunodeficiency virus type 2 /MT-4	>200
Herpes simplex virus type 1 (KOS)/E_6SM	200
Herpes simplex virus type 1 (TK^-)/E_6SM	≥200
Herpes simplex virus type 2 (G)/E_6SM	300
Vaccinia virus/E_6SM	>400
Vesicular stomatitis virus/E_6SM	400
Moloney murine sarcoma virus/C3H/3T3	>100

CYTOSTATIC ACTIVITY	50% Inhibitory concentration (μg/ml) [b])
Assay system	(β-3)
L1210	>100
CEM	>100
MT-4	>100

[a]) Concentration required to inhibit virus-induced cytopathicity by 50%. The values for the minimum cytotoxic concentration, required to cause a microscopically detectable alteration of the normal cell morphology, were for (β-2) in E_6SM, VERO and in HeLa cell cultures >200 μg/ml. The 50% cytotoxic concentration, or concentration required to reduce MT-4 cell viability by 50% was for (β-2) and >200 μg/ml.

[b]) Concentration of (β-2) required to inhibit the proliferation of murine leukemia cells (L1210) and human T-lymphoblast cells (MOLT4/clone 8 and CEM) by 50%.

CONFORMATION ANALYSIS

In view of the close relationship between conformation and biological activity (see e.g. lit.,[21]) a conformation analysis of compound (β-2) was done. Haasnoot et al.[22-24] elaborated in the past a more general Karplus equation (1):

$$^3J(H_k,H_l) = P_1\cos_2\Phi_{kl} + P_2\cos\Phi_{kl} + P_3 + \Sigma\Delta\chi_i[P_4 + P_5\cos^2(\xi\Phi_{ij} + P_6|\Delta\chi|)] \qquad (1)$$

with : $\Delta\chi_i = \Delta\chi^{\alpha}{}_i - P_7\Sigma\Delta\chi^{j\beta}$

ξ = +1 or -1

For the CH_2-CH_2-entity two sets of parameters were used :

	P_1	P_2	P_3	P_4	P_5	P_6	P_7
Set A	13.70	-0.73	0	0.56	-2.47	16.9	0.14
Set B	13.89	-0.98	0	1.02	-3.40	14.9	0.24

Both parameter sets gave roughly the same result, well within the experimental error margin. The results

are summarized in Table 4.

Table 4: Conformational parameters of the sugar puckering of compound (β-2)

	X_N	Φ_m	P_N	P_S	K	ΔG°(kJ/mol)
Set A	0.45	39.5°	26.5°($^3T_4$)	155.0°($^2T_1$)	1.22	-0.5
Set B	0.45	40.5°	26.5°($^3T_4$)	154.8°($^2T_1$)	1.22	-0.5

Haasnoot et al.[22] suggested not to take into account the experimental values of J(Hα-C(2'),Hβ-C(3')) and J(Hβ-C(2'),Hα-C(3')). Hereby a far better 'fit' between experimental and calculated coupling constants was obtained (RMS (root mean square) value 0.2 Hz vs 0.8 Hz). From Table 4 we can conclude that there exists an equilibrium between a $^3T_4$ (N-type) and a $^2T_1$ (S-type) conformation with a slight predominance of the S-form. This conformation is different from the one obtained for 2-β-D-ribofuranosylpyridine-4-carboxamide (1) (P_N = 1.2° ($^3T_2$), P_S = 183.0° ($^2T_3$), K = 1.5).

The conformation around the C-4',C-5'-bond can be calculated using two methods (Method 1,[25] Method 2[26]). Again a comparison with the values obtained for compound (1) is made. The result of these calculations is reproduced in Table 5. From these data it can be seen that the gg-rotamer is predominant in both cases, but less favored in compound (2).

Table 5: Conformational parameters around the C-4',C-5'-bond for compounds (1) and (β-2)

	Method 1[25]			Method 2[26]		
	%gg	%gt	%tg	%gg	%gt	%tg
(1)	63	25	12	58	32	10
(2)	42	32	26	40	34	26

Since removal of both hydroxyl groups resulted in the loss of all biological activity, the modified

conformation can be responsible for this effect.

EXPERIMENTAL

General. ^{1}H Nmr spectra were recorded on a Bruker-500 (500 MHz, R.U. Ghent, Ghent, Belgium) and on a Varian 400 nmr spectrometer (400 MHz, RUCA, University of Antwerp, Antwerp, Belgium). ^{13}C Nmr spectra were recorded on a Bruker WH-500 (125 MHz, R.U.Ghent, Ghent, Belgium) for compounds (α-2) and (β-2) and on a Varian 400 nmr spectrometer (100 MHz, RUCA, University of Antwerp, Antwerp, Belgium). All nmr spectra were recorded in $CDCl_3$ (TMS as internal reference), except for the ^{13}C nmr spectra of compounds (α-2) and (β-2) which were recorded in D_2O (DMSO-d_6 as external standard), the ^{1}H nmr spectra of compounds (α-2) and (β-2), recorded in CD_3OD/DMSO-d_6 (1/1), without addition of internal reference). DCI-mass spectra were obtained on a Ribermag-10-10B (Nermag S.A.) quadrupole mass spectrometer. Primary ionisation of the reagent gas (NH_3) was done with 70 eV electrons. The ionisation current was 0.08 mA and the pressure in the ion source was 0.1 mmHg.

Hplc analyses and semipreparative purifications were carried out on a Hewlett-Packard HP-1084-B hplc apparatus (resp. columns : Lichrosorb 10 RP 8 (25 cm x 4.6 mm ID) and Lichrosorb 10 RP 8 (25 cm x 22.5 mm ID)). The compounds were detected by uv (λ_{max} = 270 nm). Melting points were determined on a Leitz-Wetzlar melting point microscope and were uncorrected. For the 2-D NOESY experiment we used the sequence proposed by Macura and co-workers[27] 90°(^{1}H)-t_1-90°(^{1}H)-τ_m-$\chi(t_1)$-90°(^{1}H)-Acq. We used a relaxation delay of 1.5 sec, a 90° (^{1}H) of 5.0 µsec, a mixing time τ_m of 120 msec randomly varied by 15% in order to suppress the zero quantum J cross peaks. A matrix of 1K x 0.5K data points were obtained using 16 scans. A 45° shifted sine bell function was used in both directions.

2-(5-O-[(Tert-butyl)diphenylsilyl]-2,3-dideoxy-1-hydroxy-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (5). In a three-necked flask of 250 ml, equipped with a $CaCl_2$-tube, a dropping funnel and a gas inlet tube, 0.51 g (2 mmol) of 2-bromo-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine was dissolved in 40 ml of dry THF (freshly distilled from $LiAlH_4$). Prior to use the flask was

carefully dried and flushed with dry N_2 gas. The solution was cooled to -78 °C with a dry ice/acetone bath and 1.1 eq of butyllithium was added with the aid of a microsyringe. The solution immediately turned red. After 3 min a solution of dry THF containing 0.708 g (2 mmol) of 5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-D-ribono-1,4-lactone (3) was slowly added. After 2 h at -78 °C, the reaction mixture was allowed to come to room temperature. The reaction mixture was quenched by the addition of 80 ml of water. The aqueous phase was extracted with AcOEt (3 x 50 ml) and the combined organic layers were dried on magnesium sulfate and filtered off. After evaporation of the AcOEt, a dark yellow syrup was obtained, which was purified by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254; binder : $CaSO_4$; eluant : AcOEt; flow rate=5 ml/min) and 2-(5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-1-hydroxy-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (5) was collected (R_F=0.75; 0.58 g (55%)). ^{1}H Nmr ($CDCl_3$) : Table 1. ^{13}C Nmr ($CDCl_3$) δ values of (5) (ppm): 159.8 (C=N (oxazole)), 153.8 (C(2)), 149.1 (C(6)), 136.5 (C(4)), 135.4 (C_o (Ph)), 133.0 (C_{ipso} (Ph)), 129.6 (C_p (Ph)), 127.6 (C_m (Ph)), 124.9 (C(5)), 120.1 (C(3)), 104.6 (C(1')), 79.3 (CH_2 (oxazole)), 71.2 (C(4')), 68.0 (-C= (oxazole)), 67.7 (C(5')), 33.9 (C(2')), 28.0 (CH_3 (tert-butyl)), 27.2 (C(3')), 26.7 (CH_3 (oxazole)), 19.1 (-C= (tert-butyl)). DCI-ms (NH_3): 531 ([MH^+], 87.8), 513 ([MH^+]-H_2O, 100). Anal. Calcd for $C_{31}H_{38}N_2O_4Si$: C 70.19 H 7.17 N 5.28 : Found : C 69.23 H 7.36 N 5.62.

α/β 2-(5-O-[(Tert-butyl)diphenylsilyl]-2,3-dideoxy-1-O-mesyl-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (6). A 100 ml flask was filled with a solution of 0.106 g (0.2 mmol) of 2-(5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-1-hydroxy-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (5) in 25 ml of dry pyridine (freshly distilled from CaH_2) and an excess (0.050 g) of methanesulfonyl chloride. The reaction mixture was stirred at room temperature. After 24 h, the mixture was poured into 250 ml of a saturated $NaHCO_3$ solution. The aqueous layer was extracted with AcOEt (3 x 50 ml) and the combined organic layers were dried on magnesium sulfate. After filtration, the solvent was evaporated under vacuum, giving a dark brown foam. This foam was purified by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254, binder : $CaSO_4$; eluant : AcOEt; flow rate=5 ml/min). After evaporation of the solvent, the mesylate (6) was

isolated as a light yellow syrup (R_F=0.70, 0.097 g (80%)). ^{1}H Nmr ($CDCl_3$) : Table 1. ^{13}C Nmr ($CDCl_3$) δ values of (α-$MeSO_2$-6) (ppm): 161.1 (C=N (oxazole)), 160.6 (C(2)), 149.8 (C(6)), 135.7 (C(4), C_o (Ph)), 133.9 (C_{ipso} (Ph)), 129.6 (C_p (Ph)), 127.8 (C_m (Ph)), 121.1 (C(5)), 119.6 (C(3)), 109.2 (C(1')), 80.2 (C(4')), 79.5 (CH_2 (oxazole)), 68.2 (-C= (oxazole)), 67.4 (C(5')), 49.9 (C(2')), 38.1 (CH_3 ($MeSO_2$)), 28.3 (CH_3 (tert-butyl)), 27.6 (C(3')), 26.9 (CH_3 (oxazole)), 19.3 (-C= (tert-butyl)). δ Values of (β-$MeSO_2$-6) (ppm): 161.1 (C=N (oxazole)), 160.6 (C(2)), 149.7 (C(6)), 135.8 (C_o (Ph)), 135.7 (C(4)), 133.9 (C_{ipso} (Ph)), 129.6 (C_p (Ph)), 127.8 (C_m (Ph)), 121.2 (C(5)), 119.8 (C(3)), 109.5 (C(1')), 82.2 (C(4')), 79.4 (CH_2 (oxazole)), 68.2 (-C= (oxazole)), 66.1 (C(5')), 49.8 (C(2')), 38.2 (CH_3 ($MeSO_2$)), 28.3 (CH_3 (tert-butyl)), 26.9 (CH_3 (oxazole)), 26.6 (C(3')), 19.3 (-C= (tert-butyl)). DCI-ms (NH_3): 609 ([MH^+], 14.2), 513 ([MH^+]-CH_3SO_2H, 100). Anal. Calcd for $C_{32}H_{40}N_2O_6SSi$: C 63.16 H 6.58 N 4.61 : Found : C 62.56 H 6.32 N 4.89.

α/β **4-(4,5-Dihydro-4,4-dimethyloxazol-2-yl)-2-(5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-D-ribofuranosyl)pyridine (7).** In a three-necked flask of 250 ml, equipped with a $CaCl_2$-tube, a dropping funnel and a gas inlet tube, 27 mg (0.7 mmol) of $LiAlH_4$ was dissolved in 30 ml of dry ether (freshly distilled from Na wire). The whole apparatus was flushed with N_2 gas during 5 min. Then a solution of 61 mg (0.1 mmol) of 2-(5-O-tert-butyldiphenylsilyl-2,3-dideoxy-1-O-mesyl-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (6) was slowly added. After 1 min of additional stirring, the reaction mixture was quenched with 25 ml of water. When H_2 gas evolution had ceased, 50 ml of CH_2Cl_2 was added and the mixture was filtered to eliminate the LiAl salts. The aqueous phase was extracted later and the combined organic layers were dried on magnesium sulfate, filtered off and evaporated under reduced pressure. The resulting yellow syrup was then purified by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254, binder : $CaSO_4$; eluant : AcOEt; flow rate=5 ml/min) and the α/β-mixture of 4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)-2-(5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-D-ribofuranosyl)pyridine (7) was obtained after evaporation of the solvent (R_F=0.45, 26 mg (51%)). ^{1}H Nmr ($CDCl_3$) δ values of (α-7) (ppm): 8.61 (d, J(5,6)=5.1 Hz, 1 H, H-C(6) (Py)), 7.94 (br s, 1 H, H-C(3) (Py)), 7.60-7.75 (m, 5 H, H_o (Ph), H-C(5) (Py)), 7.35-7.45 (m, 6 H, H_m (Ph), H_p (Ph)),

5.15 (br t, J(1',2'a)=7.0 Hz, J(1',2'b)= 7.0 Hz, 1 H, H-C(1')), 4.42 (m, 1 H, H-C(4')), 4.00 (s, 2 H, CH_2 (oxazole)), 3.76 (d, J values could not be determined due to δ(H-C(5'a))=δ(H-C(5'b)), 2 H, H-C(5'a), H-C(5'b)), 2.0-2.5 (m, 3 H, H_a-C(2'), H_b-C(2'), H_a-C(3')), 1.95 (m, 1 H, H_b-C(3')), 1.40 (s, 6 H, CH_3 (oxazole)), 1.10 (s, 9 H, CH_3 (tert-butyl)). δ Values of (β-7) (ppm): 8.51 (d, J(5,6)=5.0 Hz, 1 H, H-C(6) (Py)), 7.96 (br s, 1 H, H-C(3) (Py)), 7.60-7.75 (m, 5 H, H_o (Ph), H-C(5) (Py)), 7.35-7.45 (m, 6 H, H_m (Ph), H_p (Ph)), 4.99 (t, J(1',2'a)=7.2 Hz, J(1',2'b)=7.3 Hz, 1 H, H-C(1')), 4.20(m, J(3'a,4')=6 Hz, J(3'b,4')=6 Hz, 1 H, H-4'), 4.00 (s, 2 H, CH_2 (oxazole)), 3.77 (dd, J(4',5'a)=4.7 Hz, J(5'a,5'b)=-10.7 Hz, 1 H, H-5'a), 3.73 (dd, J(4',5'b)=4.6 Hz, J(5'a,5'b)=-10.7 Hz, 1 H, H-5'b), 2.36 (m, J(2'a,2'b)=-13.3 Hz, J(2'a,3'a)=4.0 Hz, J(2'a,3'b)=6.9 Hz, 1 H, H_a-C(2')), 2.02 (m, 1 H, H_a-C(3')), 1.90 (m, 1 H, H_b-C(2')), 1.85 (m, 1 H, H_b-C(3')), 1.40 (s, 6 H, CH_3 (oxazole)), 1.10(s, 9 H, CH_3 (tert-butyl)). ^{13}C Nmr ($CDCl_3$) δ values of (α-7) (ppm): 164.8 (C=N (oxazole)), 160.3 (C(2)), 148.6 (C(6)), 137.4 (C(4)), 135.7 (C_o (Ph)), 133.7 (C_{ipso} (Ph)), 129.9 (C_p (Ph)), 127.8 (C_m (Ph)), 121.0 (C(5)), 118.9 (C(3)), 83.8 (C(4')), 79.5 (CH_2 (oxazole)), 79.3 (C(1')), 68.1 (-C= (oxazole)), 65.4 (C(5')), 33.7 (C(2')), 28.3 (CH_3 (oxazole)), 26.9 (C(3')), 26.8 (CH_3 (tert-butyl)), 19.3 (-C= (tert-butyl)). δ Values of (β-7) (ppm): 164.8 (C=N (oxazole)), 163.6 (C(2)), 149.0 (C(6)), 135.9 (C(4)), 135.7 (C_o (Ph)), 133.7 (C_{ipso} (Ph)), 129.9 (C_p (Ph)), 127.8 (C_m (Ph)), 120.6 (C(5)), 118.6 (C(3)), 81.9 (C(4')), 80.7 (C(1')), 79.5 (CH_2 (oxazole)), 68.1 (-C= (oxazole)), 66.3 (C(5')), 33.0 (C(2')), 28.3 (CH_3 (oxazole)), 28.0 (C(3')), 26.9 (CH_3 (tert-butyl)), 19.3 (-C= (tert-butyl)). *DCI-ms (NH_3): 515 ([MH^+]). Anal. Calcd for $C_{31}H_{38}N_2O_3Si$: C 72.37 H 7.39 N 5.45 : Found* : C 73.17 H 7.23 N 5.70.

α/β 4-Carbamoyl-2-(2,3-dideoxy-D-ribofuranosyl)pyridine (2). In a flask of 100 ml, 100 mg (0.20 mmol) of α/β 4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)-2-[5-O-[(tert-butyl)diphenylsilyl]-2,3-dideoxy-D-ribofuranosyl]pyridine (7) was dissolved under stirring in 50 ml of CF_3COOH/H_2O (4/1). The reaction mixture was warmed up to 60 °C. After 20 min, the mixture was poured into 200 ml of water and the aqueous layer was washed with chloroform (3 x 50 ml). After evaporation of the aqueous layer, a brown syrup was obtained. The syrup was dissolved in MeOH and neutralised with 25% of NH_4OH. The solvent was removed under reduced pressure and the resulting syrup was transferred to a reaction vessel and 100

ml of cold, saturated methanolic ammonia (-10 °C) were added. Then the vessel was carefully closed and heated to 60 °C. After 1 week, the vessel was opened and the reaction mixture was evaporated under reduced pressure. The resulting syrup was dissolved in H_2O and the solution was adjusted to pH=7 by adding 20% HCOOH. The solution was partially evaporated and purified by hplc on a Lichrosorb 10 RP 8 column (25 cm x 22.6 mm ID, eluant MeOH/H_2O (15/85), uv-detection, λ_{max}=275 nm, flow rate = 8 ml/min) by which the two anomers were separated (28.1 mg (65%)). ^{1}H Nmr (CD_3OD/DMSO-d_6) : Table 2. ^{13}C Nmr (D_2O) δ values of (α-2) (ppm): 167.9 (C=O), 164.4 (C(2)), 149.9 (C(6)), 142.9 (C(4)), 120.8 (C(5)), 118.6 (C(3)), 81.9/81.7 (C(1')/ C(4')), 64.7 (C(5')), 33.8 (C(2')), 27.6 (C(3')). δ Values of (β-2) (ppm) 168.0 (C=O), 164.7 (C(2)), 150.0 (C(6')), 142.9 (C(4)), 120.7 (C(5)), 118.2 (C(3)), 81.7/81.4 (C(1')/C(4')), 64.7 (C(5')), 33.8 (C(2')), 28.2 (C(3')). DCI-ms (NH_3): 223 ([MH^+]). Anal. Calcd for $C_{11}H_{14}N_2O_3$: C 59.46, H 6.31, N 12.61 : Found : C 58.95, H 6.40, N 12.64.

ACKNOWLEDGEMENT

P. Joos is a post-doctoral research fellow of the N.F.W.O. We thank J. Schrooten and Mr. J. Verreydt for technical assistance. We thank Prof.Dr. R. Dommisse for the use of the 400 MHz nmr apparatus.

REFERENCES

1. D. A. Cooney, H. N. Joyaram, R. I. Glazer, J. A. Kelley, V. E. Marquez, G. Gebeyehu, A. C. Van Cott, L. A. Zwelling, and D. G. Johns, Adv. Enzym., 1983, **21**, 271.
2. P. E. Joos, E. L. Esmans, R. A. Dommisse, W. F. Van Dongen, J. A. Lepoivre, F. C. Alderweireldt, J. Balzarini, and E. De Clercq, Nucleos. Nucleot., 1991, **10**, 853.
3. M. M. Kabat, K. W. Pankiewicz, and K. A. Watanabe, J. Med. Chem., 1987, **30**, 924.
4. H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. StClair, S. Nusinoff Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder, Proc. Natl. Acad. Sci. USA, 1985, **82**, 7096.

5. M. Fischl, D. Richman, M. Grieco, M. Gottlieb, P. Volberding, O. Laskin, J. Leedom, J. Groopman, D. Mildvan, R. Schooley, G. Jackson, D. Durack, and D. King, New England J. Med., 1987, **317**, 185.

6. H. Mitsuya and S. Broder, Proc. Natl. Acad. Sci. USA, 1986, **83**, 1911.

7. R. Yarchoan, R. V. Thomas, J.-P. Allain, N. McAtee, R. Dubinsky, H. Mitsuya, T. Lawley, B. Safai, C. Myers, C. Perno, R. Klecker, R. Wills, M. Fischl, M. McNeely, J. Pluda, M. Leuther, J. Collins, and S. Broder, Lancet, 1988, i, 76.

8. R. Yarchoan, H. Mitsuya, R. Thomas, J. Pluda, N. Hartman, C. Perno, K. Marczyk, J.-P. Allain, D. Johns, and S. Broder, Science, 1989, **245**, 412.

9. M. J. Robins and R. K. Robins, J. Am. Chem. Soc., 1964, **86**, 3585.

10. M. J. Robins, J. R. McCarthy, and R. K. Robins, Biochemistry, 1966, **5**, 224.

11. J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel, and I. L. Klundt, J. Org. Chem., 1966, **31**, 205.

12. J. P. Horwitz, J. Chua, M. Noel, and J. T. Donatti, J. Org. Chem., 1967, **32**, 817.

13. C. K. Chua, R. Raghavacheri, J. Warren Beach, Y. Kosugi, and G. V. Ullas, Nucleos. Nucleot., 1984, **8**, 903.

14. R. Shapiro and R.W. Chambers, J. Am. Chem. Soc., 1961, **83**, 1961.

15. M.P. Mertes, J. Zielinski, and C. Pillar, J. Med. Chem., 1967, **10**, 320.

16. D. H. R. Barton and S. W. McCombie, J. Chem. Soc., Perkin Trans. I, 1975, 1574.

17. E. J. Corey and R. A. E. Winter, J. Am. Chem. Soc., 1963, **85**, 2677.

18. M. M. Kabat, K. W. Pankiewicz, E. Sochacka, and K. A. Watanabe, Chem. Pharm. Bull., 1988, **36**, 634.

19. J. A. Piccirilli, T. Krauch, L. J. MacPherson, and S. A. Benner, Helv. Chim. Acta, 1991, **74**, 397.

20. P. E. Joos, E. L. Esmans, R. A. Dommisse, J. A. Lepoivre, F. C. Alderweireldt, A. De Bruyn, J. Balzarini, and E. De Clercq, Helv. Chim. Acta, 1992, **75**, 1613.

21. P. Van Roey, J. M. Salerno, C. K. Chu, and R. F. Schinazi, Proc. Natl. Acad. Sci. USA, 1989, **86**, 3929.

22. C. A. G. Haasnoot, F. A. A. M. de Leeuw, H. P. M. de Leeuw, and C. Altona, Org. Magn. Res., 1982, **15**, 43.

23. C. A. G. Haasnoot, F. A. A. M. de Leeuw, and C. Altona, Tetrahedron, 1980, **36**, 2783.

24. F. A. A. M. de Leeuw, and C. Altona, J. Chem. Soc., Perkin Trans. II, 1982, 375.

25. D. B. Davies and A. Rabczenko, J. Chem. Soc., Perkin Trans. II, 1975, 1703.

26. C. A. G. Haasnoot, F. A. A. M. de Leeuw, H. P. M. de Leeuw, and C. Altona, Rec. Trav. Chim. Pays-Bas, 1979, **98**, 576.

27. S. Macura, K. Wütrich, and R. R. Ernst, J. Magn. Reson., 1982, **46**, 269.

Received, 20th June, 1995