SYNTHESIS, CONFORMATIONAL ANALYSIS AND BIOLOGICAL EVALUATION OF 2-(5-DEOXY-β-D-RIBOFURANOSYL)PYRIDINE-4-CARBOXAMIDE

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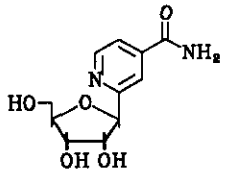
Abstract - Coupling of 5-deoxy-2,3-O-isopropylidene-D-ribono-1,4-lactone (5) with 2-lithio-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (6), subsequent mesylation, followed by reduction and ammonolysis resulted in the formation of 2-(5-deoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (4). The anomeric configuration was established with 2D-NOESY-^{1}H-nmr-spectroscopy. Conformational analysis was done with the aid of the high resolution ^{1}H-nmr-spectroscopy data. Compound (4) possessed modest cytostatic activity against a number of tumor cell lines and was slightly inhibitory to vaccinia virus and vesicular stomatitis virus.

INTRODUCTION

In a previous paper[1] it was demonstrated that 4-carbamoyl-2-β-D-ribofuranosylpyridine (1) had a modest inhibitory effect on the growth of certain tumor cell lines, whereas for the 2'-deoxy- (2)[2] and the 2',3'-dideoxy-derivative (3),[3] no biological activity was observed. Therefore, within the scope of our in vitro structure-activity-relation studies (Figure 1), 2-(5-deoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (4) was synthesized.

RESULTS AND DISCUSSION

In the literature 5'-deoxynucleosides are synthesized according to a variety of procedures.[4-7] All those pathways start from the ribonucleosides. Since all these methods are non-branched pathways, a new method was elaborated based upon the use of 5-deoxy-2,3-O-isopropylidene-D-ribono-1,4-lactone (5) (Scheme 1). The synthesis of the latter compound is described in the literature.[8] However we preferred a modified procedure starting from D-ribono-1,4-lactone.



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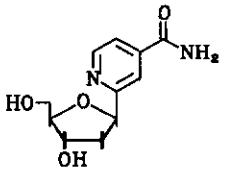

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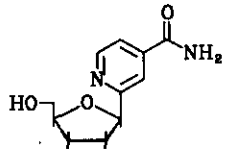

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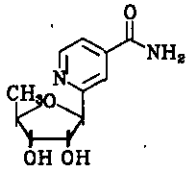
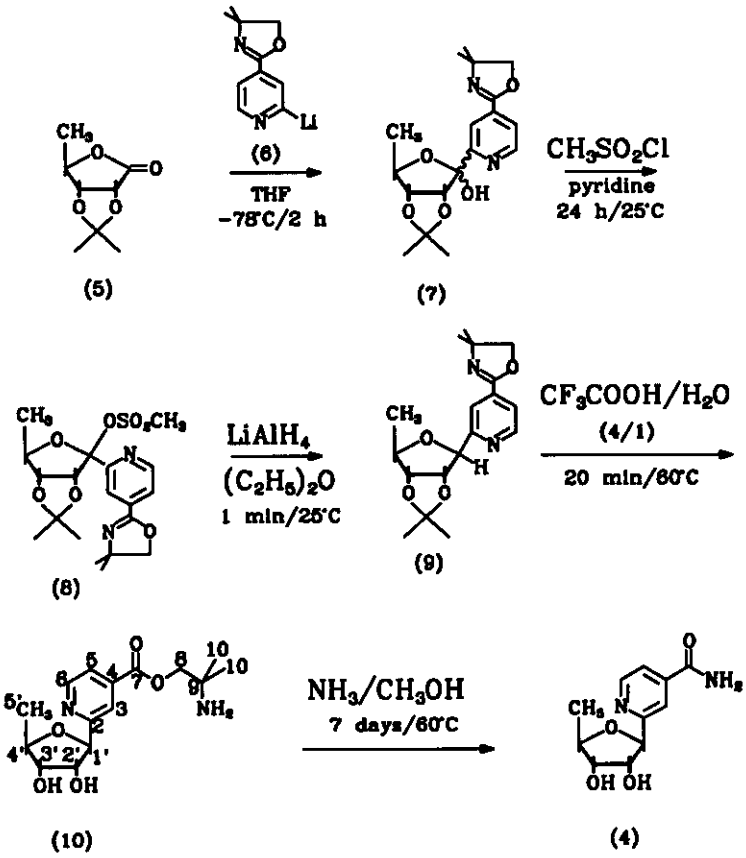


(4)

Figure 1: In vitro SAR-study of pyridine-C-nucleosides

In this procedure the 2- and 3-hydroxyl groups were protected as an isopropylidene group, using the procedure of Hough et al.[9] The 2,3-O-isopropylidene derivative was obtained in 62% yield. Treatment

of 2,3-O-isopropylidene-D-ribono-1,4-lactone with methanesulfonyl chloride in pyridine gave the corresponding 5-O-mesylate in 85 % yield. Refluxing with LiCl in acetone gave the 5-chloro compound (90 % yield), which by the action of Bu_3SnH/AIBN in toluene gave the desired 5-deoxy-2,3-O-isopropylidene-D-ribono-1,4-lactone (5) (75 % yield). The spectroscopic data of the latter compound were in agreement with the literature data.[8]



Scheme 1: Synthesis of 2-(5-deoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (4)

2-Lithio-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (6) could be 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.[1,2] To this solution, a solution of 5-deoxy-2,3-O-isopropylidene-D-ribono-1,4-lactone (5)

was added and after the usual work-up compound (7) was obtained as an anomeric mixture of lactols in a ratio 60/40 as determined by integration of the H-C(3)-proton in the 400 MHz ^{1}H-nmr spectrum.

Table 1: 400 MHz ^{1}H-Nmr data of compounds α-OH-(7), β-OH-(7) and (8).

Compound	α-OH-(7) ($CDCl_3$/TMS)	β-OH-(7) ($CDCl_3$/TMS)	(8) ($CDCl_3$/TMS)
H-C(2')	5.02 (d)	4.73 (d)	5.89 (d)
H-C(3')	4.42 (dd)	4.76 (dd)	4.99 (dd)
H-C(4')	4.32 (dd)	4.55 (dd)	4.49 (dd)
H-C(5')	1.30 (d)	1.42 (d)	1.26 (d)
H-C(3)	8.08 (br s)	8.05 (br s)	8.47 (br s)
H-C(5)	7.66 (dd)	7.70 (dd)	7.96 (dd)
H-C(6)	8.58 (dd)	8.48 (dd)	8.71 (dd)
CH_3a (isoprop.)	1.40 (s)	1.18 (s)	1.42 (s)
CH_3b (isoprop.)	1.58 (s)	1.27 (s)	1.60 (s)
CH_2 (oxazole)	4.03 (s)	4.03 (s)	4.11 (s)
CH_3 (oxazole)	1.29 (s)	1.29 (s)	1.34 (s)
CH_3 ($MeSO_2$)[a])	-	-	2.69 (s)
J(2',3')	7.3 Hz	5.7 Hz	7.5 Hz
J(3',4')	4.6 Hz	1.4 Hz	4.0 Hz
J(4',5')	6.4 Hz	7.0 Hz	6.3 Hz
J(3,5)	1.5 Hz	1.5 Hz	1.7 Hz
J(3,6)	0.9 Hz	0.9 Hz	0.9 Hz
J(5,6)	5.0 Hz	5.2 Hz	5.0 Hz

[a]) $MeSO_2$= OSO_2CH_3

In the 400 MHz ^{1}H-nmr spectrum two sets of isopropylidene signals were observed separated by respectively 0.18 and 0.09 ppm. Applying the 'Imbach rule' for C-nucleosides[10] the value of $\Delta\delta(CH_3)$ of 0.09 ppm was assigned to the β-OH anomer (major compound) and the value of $\Delta\delta(CH_3)$ 0.18 ppm to the α-anomer. Although these results are in agreement with other literature data,[11,12] we want to

emphasize that this rule should be handled with care since it was designed for nucleosides and not for lactols.

Surprisingly treatment of these lactols with methanesulfonyl chloride in dry pyridine gave only one isomer of the mesylate (8) (80% yield). In its ^{1}H-nmr spectrum a $\Delta\delta(CH_3)$-value of 0.18 ppm was measured suggesting a β-OSO_2CH_3 compound. Further investigation of its nmr spectrum showed that H-C(2') was shifted 0.8 ppm downfield. This phenomenon was already observed by Watanabe and coworkers[13] on acetalisation of the OH-C(1')-function of 2-bromo-6-(1-hydroxy-2,3-O-isopropylidene-5-O-tetrahydropyranyl-D-ribofuranosyl)pyridine. This gave a downfield shift of 0.4 ppm. This shift was explained by assuming that both the 1'-O-acetyl group and H-C(2') were on the same side of the ring, which is a β-configuration for the 1'-O-acetyl function. Another argument for the β-OSO_2CH_3 configuration of compound (8) is based on stereoelectronic arguments. Ohrui et al.[14,15] stated that substituents like -OR or halogenides in 1'-position in 2,3-O-isopropylidene furanoses tend to adopt a trans-position with respect to the isopropylidene function. Therefore it is reasonable to assume that upon mesylation the hemiacetal isomerizes to its prefered β-configuration. For the corresponding nmr data of compound (8) we refer to Table 1.

Reduction of the mesylate (8) gave the fully protected nucleoside (9) in reasonable yields (47%), which was deprotected using CF_3COOH/H_2O (4/1) at 60°C. This resulted in the removal of the isopropylidene moiety and opening of the 4,5-dihydrooxazole ring to the corresponding aminoester (10).

Assignment of the anomeric configuration of 10 was performed with 2D-NOESY ^{1}H-nmr spectroscopy (Figure 2).

In the spectrum cross-peaks were observed between H-C(1') and H-C(4') proving the expected β-configuration as well as the usual cross-peaks between H-C(2'), H-C(3') and H-C(5') (Figure 2). The cross-peaks between H-C(5') and H-C(6) is probably due to the anti-position around the glycosidic bond. Diffusion effects are responsible for the cross-peaks between H-C(5) and H-C(6).

Subsequent ammonolysis of 10 gave the corresponding amide (4), which was purified using CCTLC (CHROMATOTRON^R, eluant CH_2Cl_2/CH_3OH 85/15). The high resolution ^{1}H-nmr-data are depicted in

Table 2. Numbering of the protons is as in Scheme 1.

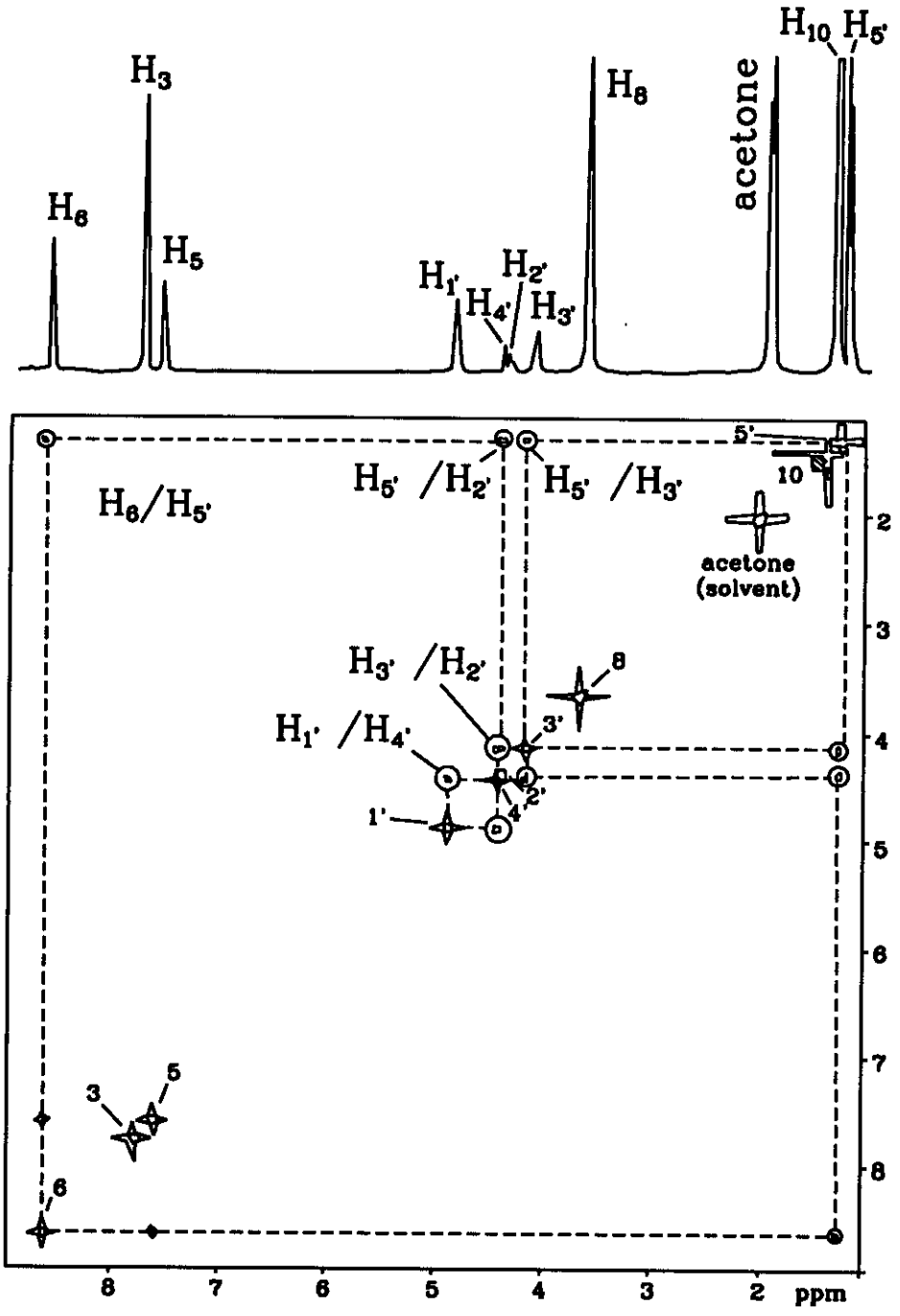


Figure 2: NOESY-spectrum of compound (10)

Table 2: ^{1}H-Nmr data of compound (4).

Compound	(4) (CD_3OD/ DMSO-d_6 (1/1))
H-C(3)	7.88 (br s)
H-C(5)	7.71 (dd)
H-C(6)	8.63 (d)
H-C(1')	4.86 (d)
H-C(2')	4.30 (dd)
H-C(3')	4.06 (dd)
H-C(4')	4.46 (dd)
H-C(5')	1.33 (d)
J(3,5)	1.8 Hz
J(5,6)	5.0 Hz
J(1',2')	8.7 Hz
J(2',3')	4.4 Hz
J(3',4')	3.4 Hz
J(4',5')	6.6 Hz

CONFORMATIONAL ANALYSIS

The ^{1}H-nmr data were used to calculate the different pseudorotation parameters. Since no specific equations for 5'-deoxy-D-ribofuranosyl nucleosides were available, we neglected the effect of a group in 5'-position and we used the parameter set used by Altona and coworkers.[16] Via an iterative computer programme the pseudorotation parameters were obtained. They are summarized in Table 3. The corresponding calculated coupling constants gave a good 'fit' with the experimental ones (RMS=0.052 Hz).

Table 3: Pseudorotational parameters of compound (4)

X_N	Φ_m	P_N	P_S	K	ΔG (kJ/mol)
0.10	55.2	-4.5 ($_2T^3$)	133 ($_1T^2$)	9	-5.0

BIOLOGICAL STUDIES

Compound (4) was screened on his antiviral and cytostatic properties (Table 4). It showed a modest cytostatic activity against a number of cell tumor lines and proved slightly inhibitory to vaccinia virus and vesicular stomatitis virus replication in E_6SM cells.

Table 4: Biological evaluation of compound (4)

Antiviral activity	Minimum inhibitory concentration (g/ml) [a]
Assay system	(4)
Human immunodeficiency virus type 1/MT-4	>200
Human immunodeficiency virus type 2 /MT-4	>200
Herpes simplex virus type 1 (KOS)/E_6SM	300
Herpes simplex virus type 1 (TK^-)/E_6SM	>200
Herpes simplex virus type 2 (G)/E_6SM	>200
Vaccinia virus/E_6SM	150
Vesicular stomatitis virus/E_6SM	70
Coxsackie virus B4/VERO	>200
Polio-1 virus/HeLa	>200
Parainfluenza-3 virus/VERO	>200
Reovirus-1/VERO	>200
Sindbis virus/VERO	>200
Semliki Forest virus/VERO	>200
CYTOSTATIC ACTIVITY	50% Inhibitory concentration (g/ml) [b]
Assay system	(4)
L1210	90
CEM	82
MOLT4/clone 8	102
MT-4	>200

[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 4 in E_6SM, VERO and in HeLa cell cultures >200 g/ml. The 50% cytotoxic concentration, or concentration required to reduce MT-4 cell viability by 50% was for 4 >200 g/ml.

[b]) Concentration of 4 required to inhibit the proliferation of murine leukemia cells (L1210) and human T-lymphoblast cells (MOLT4/clone 8 and CEM) by 50%.

EXPERIMENTAL

General - ^{1}H-Nmr spectra were obtained using a Varian-400 nmr spectrometer (400 MHz, RUCA, University of Antwerp, Antwerp, Belgium). ^{13}C-Nmr spectra were recorded on the same instrument (Varian-400 : 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 4 and 10 which were recorded in D_2O (DMSO-d_6 as external standard), the ^{1}H-nmr spectra of compounds (4) and (10) (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 mm Hg. For the 2-D NOESY experiment we used the sequence proposed by Macura and coworkers[17] 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 sec, 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.

2,3-O-Isopropylidene-D-ribono-1,4-lactone. In a flask of 250 ml, 10 g (0.068 mol) of D-ribono-1,4-lactone was dissolved in 100 ml of dry acetone (freshly distilled from K_2CO_3). To this solution 1.5 ml (1.2 equiv.) of 12 N HCl were added and stirring was continued for another 4 h at room temperature. Then the reaction mixture was poured into 250 ml of a saturated $NaHCO_3$ solution and the solution was extracted with AcOEt (3 x 200 ml). The combined organic layers were dried on magnesium sulfate and filtered off. The AcOEt was evaporated under reduced pressure and the crude 2,3-O-isopropylidene-D-ribono-1,4-lactone was recrystallised from cyclohexane/acetone (10/1). (mp : 143-145 C; 7.9 g (62%)).

^{1}H-Nmr (acetone-d_6): δ values (ppm) 4.82 (d, 1H, H-C(2), J(2,3)=5.5 Hz), 4.76 (br d, 1H, H-C(3), J(3,4)<0.5 Hz), 4.58 (dd appearing as a triplet, 1H, H-C(4), J(4,5a)=J(4,5b)=2.3 Hz), 4.42 (br dd appearing as a broad triplet, 1H, OH-C(5), J(5a,CH_2OH)=4.4 Hz, J(5b,CH_2OH)=4.9 Hz), 3.82 (m, 2H, Ha-C(5), Hb-C(5)), 1.42 (s, 3H, CH_3a (isopropylidene)), 1.37 (s, 3H, CH_3b (isopropylidene)). ^{13}C-Nmr (acetone-d_6) δ values (ppm): 174.7 (C=O), 113.1 (-C= (isopropylidene)), 83.5 (C(4)), 79.4 (C(2)), 76.5 (C(3)), 62.3 (C(5)), 27.1 (CH_3a (isopropylidene)), 25.7 (CH_3b (isopropylidene)). DCI-MS(NH_3): 206 ([M+NH_4]$^+$, 100), 189 ([MH]$^+$, 15.3). Anal. Calcd for $C_8H_{12}O_5$: C 51.06, H 6.38 . Found : C 50.95, H 6.29.

2,3-O-Isopropylidene-5-O-mesyl-D-ribono-1,4-lactone. A 200 ml flask was filled with a solution of 5 g (0.027 mol) of 2,3-O-isopropylidene-D-ribono-1,4-lactone in 50 ml of dry pyridine (freshly distilled from CaH_2) and an excess (1 ml) of methanesulfonyl chloride. The reaction mixture was stirred at room temperature. After 2.5 h the mixture was poured into a 350 ml of a saturated $NaHCO_3$ solution. The aqueous layer was extracted with AcOEt (3 x 100 ml) and the combined organic layers were dried on magnesium sulfate. After filtration the solvent was evaporated under vacuum, giving a brown syrup, which was further purified by column chromatography (Kieselgel 60, particle size 0.040-0.063 mm (230-400 mesh ASTM), eluant : AcOEt) (6.0 g (85%)). ^{1}H-Nmr ($CDCl_3$) δ values (ppm): 4.84 (d, 1H, H-C(2), J(2,3)=5.7 Hz), 4.81 (d, 1H, H-C(3), J(3,4)<0.5 Hz), 4.80 (dd appearing as a triplet, 1H, H-C(4), J(4,5a)=J(4,5b)=2.5 Hz), 4.48 (dd, 2H, Ha-C(5), Hb-C(5)), 3.03 (s, 3H, CH_3 ($MeSO_2$)), 1.43 (s, 3H, CH_3a (isopropylidene)), 1.35 (s, 3H, CH_3b (isopropylidene)). ^{13}C-Nmr ($CDCl_3$) δ values (ppm): 173.4 (C(1)), 113.9 (-C= (isopropylidene.)), 79.6 (C(4)), 77.5 (C(3)), 75.1 (C(2)), 68.4 (C(5)), 37.6 (CH_3 ($MeSO_2$)), 26.7 (CH_3a (isopropylidene)), 25.5 (CH_3b (isopropylidene)). DCI-ms (NH_3): 284 ([M+NH_4]$^+$, 100), 267 ([MH]$^+$, 2.6). Anal. Calcd for $C_9H_{14}O_7S$: C 40.60, H 5.26, S 12.03 . Found : C 40.72, H 5.10.

5-Chloro-2,3-O-isopropylidene-D-ribono-1,4-lactone. A flask of 250 ml was filled with 2 g (7.5 mmol) of 2,3-O-isopropylidene-5-O-mesyl-D-ribono-1,4-lactone, an excess (1 g) of LiCl and 50 ml of dry acetone (freshly distilled from K_2CO_3). The reaction mixture was allowed to reflux during 4 h. After filtration of the Li salts, the reaction mixture was extracted with AcOEt (3 x 150 ml). The combined organic layers were dried on magnesium sulfate and filtered off. The AcOEt was evaporated under reduced pressure.

Since no impurities were observed, 5-chloro-2,3-O-isopropylidene-D-ribono-1,4-lactone was used in the next step without further purification. (1.39 g (90%)).

5-Deoxy-2,3-O-isopropylidene-D-ribono-1,4-lactone (5). In a flask of 100 ml 3 g (14.6 mmol) of 5-chloro-2,3-O-isopropylidene-D-ribono-1,4-lactone, 0.1 g (0.61 mmol) of azobisisobutyronitrile (AIBN) and 4 ml (1.1 equiv.) of tributyltin hydride in 30 ml of dry toluene (freshly distilled from Na-wire) were stirred for 18 h in an oil bath (80 C). The reaction mixture was then allowed to cool to room temperature and the toluene was evaporated under reduced pressure. The crude reaction product was dissolved in acetonitrile and the solution was washed with hexane in order to remove unreacted tributyltin hydride. The acetonitrile-layer was dried on magnesium sulfate, filtered off and evaporated under reduced pressure. The crude (5) was purified by column chromatography (Kieselgel 60, particle size 0.040-0.063 mm (230-400 mesh ASTM), eluant : AcOEt) (1.9 g (75%)). ^{1}H-Nmr ($CDCl_3$) δ values (ppm): 4.77 (d, 1H, H-C(2), J(2,3)=5.5 Hz), 4.66 (dd, 1H, H-C(4), J(3,4)=0.6 Hz, J(4,5)=7.0 Hz), 4.49 (dd, 1H, H-C(3)), 1.37 (d, 3H, H-C(5)), 1.45 (s, 3H, CH_3a (isopropylidene)), 1.36 (s, 3H, CH_3b (isopropylidene)). ^{13}C-Nmr ($CDCl_3$) δ values (ppm): 173.6 (C(1)), 113.2 (-C= (isopropylidene)), 80.0 (C(3)), 78.8 (C(4)), 74.4 (C(2)), 26.4 (CH_3a (isopropylidene)), 25.2 (CH_3b (isopropylidene)), 13.2 (C(5)). DCI-ms (NH_3): 190 ($[M+NH_4]^+$, 100). Anal. Calcd for $C_8H_{12}O_4$: C 55.81, H 6.98 . Found : C 55.62, H 7.09.

α/β 2-(5-Deoxy-1-hydroxy-2,3-O-isopropylidene-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (7). 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. (1.4 ml) of a 1.6 M solution of butyllithium in hexane was added with the aid of a microsyringe. The solution immediately turned red. After 3 min a solution of dry THF containing 0.37 g (2 mmol) of 5 was slowly added. After 2 h at -78 C the reaction mixture was brought 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. Purification of the α/β-mixture was done by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254; binder : $CaSO_4$; eluant : AcOEt; flow rate = 6 ml/min) and the anomers α/β-OH-(7) were collected together as a white foam (R_F=0.63; 0.37 g (48%)). ^{1}H-Nmr ($CDCl_3$) : Table 1. ^{13}C-Nmr ($CDCl_3$) δ values of α-OH-(7) (ppm): 160.0 (C(2)), 157.5 (C=N (oxazole)), 148.6 (C(6)), 136.1 (C(4)), 119.9 (C(5)), 116.8 (C(3)), 112.2 (-C=(isopropylidene)), 101.1 (C(1')), 85.9 (C(3')), 84.4 (C(2')), 79.0 (C(4')), 78.9 (CH_2 (oxazole)), 67.7 (-C= (oxazole)), 27.8 (CH_3 (oxazole)), 26.2 (CH_3a (isopropylidene)), 25.0 (CH_3b (isopropylidene)), 18.5 (C(5')). δ values of β-OH-(7) (ppm): 159.9 (C(2)), 157.5 (C=N (oxazole)), 147.1 (C(6)), 135.4 (C(4)), 121.3 (C(5)), 118.5 (C(3)), 112.2 (-C= (isopropylidene)), 105.6 (C(1')), 88.5 (C(2')), 86.6 (C(3')), 83.3 (C(4')), 78.9 (CH_2 (oxazole)), 67.7 (-C= (oxazole)), 27.8 (CH_3 (oxazole)), 25.9 (CH_3a (isopropylidene)), 24.4 (CH_3b (isopropylidene)), 21.3 (C(5')). DCI-ms (NH_3): 349 ([MH]$^+$, 100). Anal. Calcd for $C_{18}H_{24}N_2O_5$: C 62.07, H 6.90, N 8.05 . Found : C 61.95, H 6.75, N 8.69.

2-(5-Deoxy-2,3-O-isopropylidene-1-O-mesyl-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (8). A 100 ml flask was filled with a solution of 0.174 g (0.5 mmol) of 7 in 40 ml of dry pyridine (freshly distilled from CaH_2) and an excess (1 ml) 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.Purification was done by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254; binder : $CaSO_4$; eluant : AcOEt; flow rate = 6 ml/min) and (8) was collected as a colorless syrup (R_F = 0.85; 0.181 g (85%)). ^{1}H-Nmr ($CDCl_3$) Table 1. ^{13}C-Nmr ($CDCl_3$) δ-values of (8) (ppm): 158.6 (C=N (oxazole)), 151.5 (C(2)), 148.7 (C(6)), 136.2 (C(4)), 125.2 (C(5)), 119.8 (C(3)), 109.1 (C(1'), -C= (isopropylidene)), 79.0 (C(2')), 78.8 (CH_2 (oxazole)), 78.0 (C(3')), 76.3 (C(4')), 67.4 (-C= (oxazole)), 37.7 (CH_3 ($MeSO_2$)), 25.6 (CH_3a (isopropylidene)), 24.2 (CH_3b (isopropylidene), CH_3 (oxazole)), 22.8 (C(5')). DCI-ms (NH_3): 427 ([MH]$^+$, 100). Anal. Calcd for $C_{19}H_{26}N_2O_7S$: C 53.66, H 6.10, N 6.57, S 7.51 . Found : C 53.95, H 6.01, N 6.25.

2-(5-Deoxy-2,3-O-isopropylidene-β-D-ribofuranosyl)-4-(4,5-dihydro-4,4-dimethyloxazol-2-yl)pyridine (9). In a three-necked flask of 250 ml, equipped with a $CaCl_2$-tube, a dropping funnel and a gas inlet tube, 135 mg (3.5 mmol) of $LiAlH_4$ was dissolved in 50 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 213 mg (0.5 mmol) of 8 was slowly added. After 1 min additional stirring, the reaction mixture was quenched with 50 ml of water. When H_2-gas evolution had ceased the 50 ml of CH_2Cl_2 were added and the mixture was filtered to eliminate the Li-Al salts. The aqueous phase was extracted another time and the combined organic layers were dried on magnesium sulfate, filtered off and evaporated under reduced pressure. Purification was done by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254; binder : $CaSO_4$; eluant : AcOEt; flow rate = 6 ml/min) and 9 was collected as a white foam (R_F=0.42; 0.0747 g (47%)). ^{1}H-Nmr ($CDCl_3$) δ values of 9 (ppm): 8.57 (d, J(5,6)=5.1 Hz, 1 H, H-C(6) (Py)), 7.81 (br s, 1 H, H-C(3) (Py)), 7.61 (dd, J(3,5)=1.5 Hz, J(5,6)=5.1 Hz, 1 H, H-C(5) (Py)), 5.22 (dd appearing as a doublet, J(1',2')<0.5 Hz, J(2',3')=6.1 Hz, 1 H, H-C(2')), 5.07 (br s, 1 H, H-C(1')), 4.56 (dd, J(2',3')=6.1 Hz, J(3',4')=4.0 Hz, 1 H, H-C(3')), 4.06 (s, 2 H, CH_2 (oxazole)), 4.03 (dd, J(3',4')=4.0 Hz, J(4',5')=6.3 Hz, 1 H, H-4'), 1.51 (s, 3 H, CH_3a (isopropylidene)), 1.35 (d, J(4',5')=6.3 Hz, 1 H, H-5'), 1.32 (s, 3 H, CH_3b (isopropylidene)), 1.31 (s, 6 H, CH_3 (oxazole)). ^{13}C-Nmr ($CDCl_3$) δ values of (9) (ppm): 160.4 (C(2)), 159.9 (C=N (oxazole)), 149.7 (C(6)), 136.4 (C(4)), 120.9 (C(5)), 119.5 (C(3)), 112.6 (-C= (isopropylidene)), 86.2 (C(1')), 85.7 (C(4')), 82.7 (C(2')), 79.4 (CH_2 (oxazole)), 77.5 (C(3')), 68.2 (-C= (oxazole)), 28.3 (CH_3 (oxazole)), 26.5 (CH_3a (isopropylidene)), 25.4 (CH_3b (isopropylidene)), 14.1 (C(5')). DCI-ms (NH_3): 333 ($[MH]^+$). Anal. Calcd for $C_{18}H_{24}N_2O_4$: C 65.06, H 7.23, N 8.43 . Found : C 65.95, H 7.02, N 8.42.

2-(5-Deoxy-β-D-ribofuranosyl)pyridine-4-carboxamide (4). In a flask of 100 ml, 83 mg (0.25 mmol) of 9 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 CH_3OH and neutralised with 25% of NH_4OH. The solvent was removed under

reduced pressure. For the 2D-NOESY-spectroscopy the obtained amino ester (10) was purified on a CHROMATOTRON^R (silica gel 60 PF-254; binder : $CaSO_4$; eluant : CH_2Cl_2/CH_3OH (8/2); flow rate = 6 ml/min. Anal. Calcd for $C_{15}H_{22}N_2O_5$: C 58.06, H 7.10, N 9.03 . Found : C 57.56, H 7.25, N 8.98). In order to synthesize the amide, the resulting syrup was transferred to a reaction vessel and 100 ml of cold, saturated methanolic ammonia (-10 C) was 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. Purification was again done by centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron^R and (4) was isolated (28 mg (47%)). ^{1}H-Nmr (CD_3OD/DMSO-d_6) : Table 2. ^{13}C-Nmr (D_2O) δ values of (4) (ppm): 170.2 (C=O), 162.8 (C(2)), 150.1 (C(6)), 143.1 (C(4)), 121.4 (C(5)), 120.3 (C(3)), 83.8 (C(4')), 82.3 (C(1')), 78.8 (C(2')), 73.8 (C(3')), 15.6 (C(5')). DCI-ms (NH_3) m/z 239 ($[MH]^+$). Anal. Calcd for $C_{11}H_{14}N_2O_4$: C 55.46, H 5.88, N 11.76 . Found : C 55.30, H 6.09, N 11.70.

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