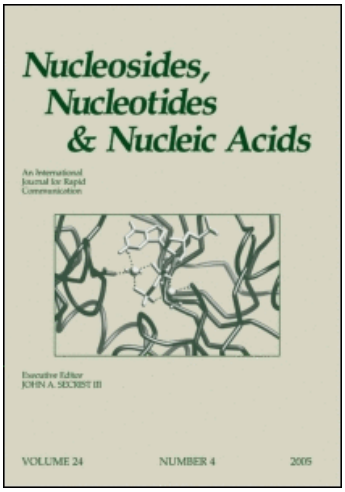

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SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-CARBAMOYL-2-β-D-RIBOFURANOSYL-PYRIDINE

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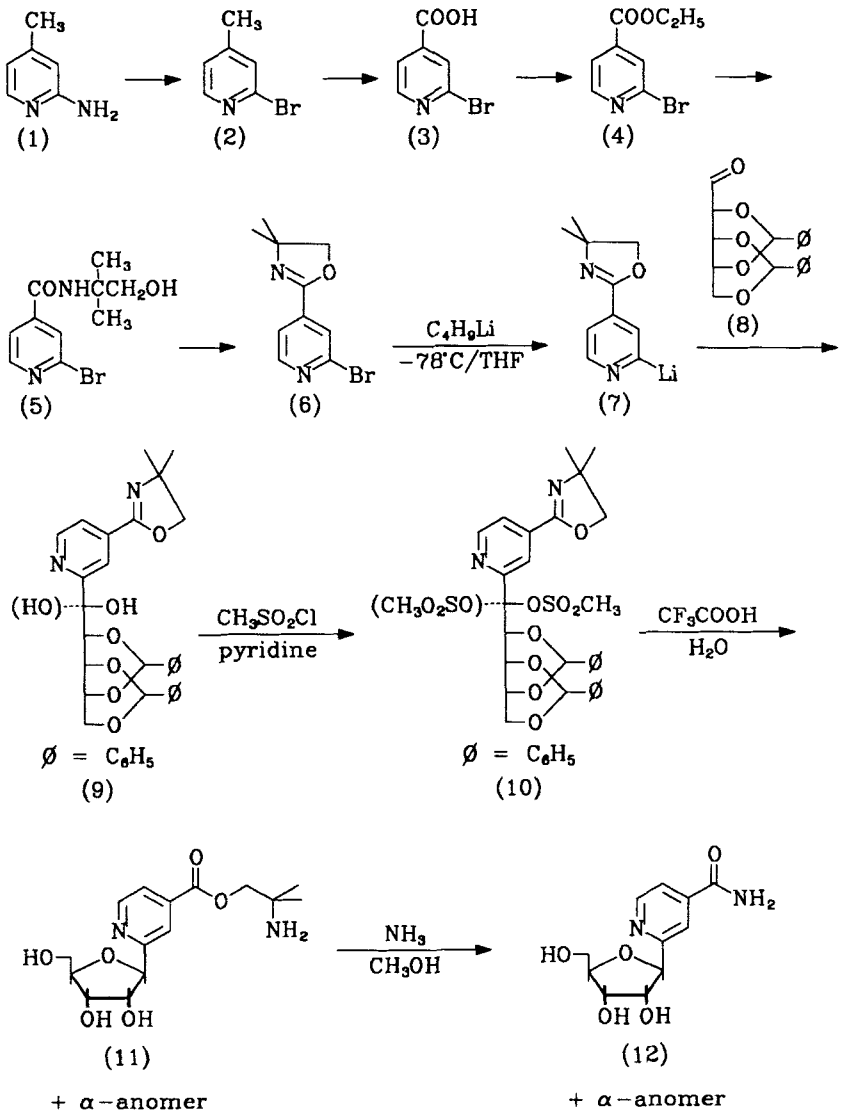
Abstract - D-Allo/D-altro 2-(2,4:3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine was synthesized from 2-lithio-4-(4,4-dimethyloxazolin-2-yl)pyridine and 2,4:3,5-di-O-benzylidenealdehydo-D-ribose. After mesylation and subsequent treatment of the adduct with CF_3COOH/H_2O and then ammonia, 4-carbamoyl-2-D-ribofuranosylpyridine was formed. The α- and β-anomers were separated by semipreparative hplc on a LICHROSORB 10 DIOL column. The β-anomer had no antiviral activity, but it had modest cytostatic activity against tumor cells.

In the context of a programme aiming at the synthesis of new pyridine-C-nucleosides with potential antiviral and/or cytostatic properties,[1-4] 4-carbamoyl-2-β-D-ribofuranosylpyridine was synthesized according to SCHEME 1.

2-Bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine (6) was prepared from 2-amino-4-methylpyridine in 4 steps : Craig diazotation[5] of 2-amino-4-methylpyridine (1) resulted in the formation of 2-bromo-4-methylpyridine (2) (83% yield). Subsequent oxidation of this compound with the aid of $KMnO_4$ gave the corresponding 2-bromopyridine-4-carboxylic acid (3) (32% yield). 2-Bromopyridine-4-carboxylic acid was converted into the ethyl ester (4) by azeotropic esterification (80% yield). Treatment of ethyl 2-bromopyridine-4-carboxylate with 2-amino-2-methylpropanol and subsequent cyclization with concentrated H_2SO_4 (110°C, 10 min) yielded the desired 2-bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine (6) in 30% yield.

2,4;3,5-Di-O-benzylidenealdehydo-D-ribose (8) was synthesized according to Zinner.[6,7]



SCHEME 1

In order to obtain the D-allo/D-altro 2-(2,4;3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine (9), 2-lithio-4-(4,4-dimethyloxazolin-2-yl)pyridine (7) was prepared in situ by adding 1.1 eq. of butyllithium to a solution of 2 eq. of 2-bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine in THF at -78°C. After 3 min 1 eq. of 2,4;3,5-di-O-benzylidenealdehydo-D-ribose in THF was slowly added. D-Allo/D-altro 2-(2,4;3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine (9)

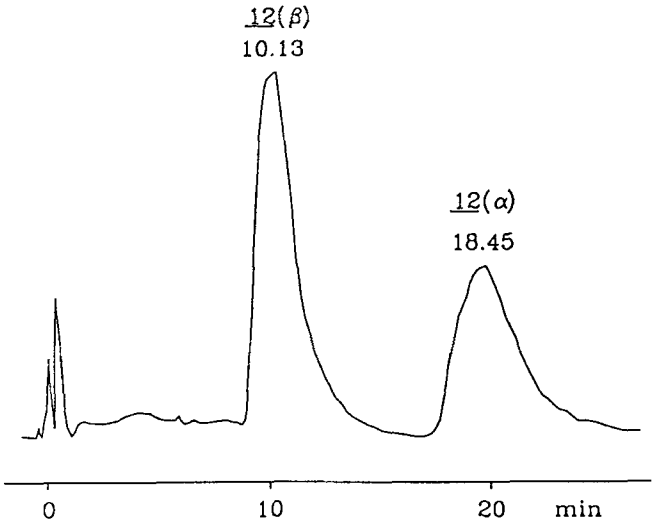


FIG. 1 : Hplc-chromatogram of the α/β-mixture of (12) : column : Lichrosorb 10 DIOL (25 cm x 4.6 mm ID), eluant : 5% 2-propanol, 95% CH_2Cl_2/CH_3COOH (99.5%-0.5%), flow-rate : 3 ml/min.

was obtained in 80% yield. Treatment of this compound with methanesulphonyl chloride in dry pyridine resulted in the formation of D-allo/D-altro 2-(1-O-mesyl-2,4;3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine (10) (83% yield). Stirring the mesylate in CF_3COOH/H_2O (4/1) for 20 min at 25°C resulted in the formation of (11). This was converted into 4-carbamoyl-2-D-ribofuranosylpyridine with saturated NH_3/CH_3OH, for 1 week (50% yield). This compound was obtained as an α/β-mixture (α/β-ratio : 44/56). Since the ring closure occurs via an S_N2-mechanism, the observed α/β-ratio in compound (12) reflects the D-allo/D-altro-ratio of (9) and (10). For the discussion of this S_N2-mechanism we refer to reference 8.

The α/β-mixture of (12) was analyzed by hplc on a LICHROSORB 10 DIOL-column (25 cm x 4.6 mm ID; flow-rate 3 ml/min; eluant : 5% 2-propanol, 95% CH_2Cl_2/CH_3COOH (99.5%-0.5%)). As depicted in FIG. 1, two components eluted with a retention time of 10.13 min and 18.45 min respectively. Analogous chromatographic conditions were applied in order to separate and isolate both anomers in a semi-preparative way. For this purpose a 25 cm x 9 mm ID LICHROSORB 10 DIOL column was used at a flow-rate of 7 ml/min. In both hplc-experiments the compounds were detected at 265 nm.

TABLE 1 : 360 MHz ^{1}H-nmr data of (12α) in CD_3OD and 500 MHz ^{1}H-nmr data of (12β) in CD_3OD/DMSO-d_6.

Compound	(12α)	(12β)
H-1'	5.044(d)	4.766(d)
$J_{1',2'}$	7.65 Hz	5.07 Hz
H-2'	4.125(dd)	4.014(t)
$J_{1',2'}$	7.65 Hz	5.07 Hz
$J_{2',3'}$	3.90 Hz	4.96 Hz
H-3'	4.255(dd)	3.888(t)
$J_{2',3'}$	3.90 Hz	4.96 Hz
$J_{3',4'}$	4.60 Hz	3.81 Hz
H-4'	4.301(m)	3.902(m)
H-5'	3.669(dd)	3.671(dd)
$J_{5',4'}$	6.60 Hz	3.15 Hz
$J_{5',5''}$	-8.46 Hz	-12.2 Hz
H-5''	3.669(dd)	3.544(dd)
$J_{5'',4'}$	6.71 Hz	4.51 Hz
$J_{5',5''}$	-8.46 Hz	-12.2 Hz
H-3	7.932(br s)	7.865(br s)
$J_{3,5}$	1.69 Hz	1.71 Hz
H-5	7.683(dd)	7.653(dd)
$J_{3,5}$	1.69 Hz	1.71 Hz
$J_{5,6}$	5.08 Hz	4.98 Hz
H-6	8.624(d)	8.630(d)
$J_{5,6}$	5.08 Hz	4.98 Hz

After evaporation of the solvent the structure of the products was investigated by 360 MHz ^{1}H-nmr spectroscopy (compound (12α)) and by 500 MHz ^{1}H-nmr spectroscopy (compound (12β)). The results are summarized in TABLE 1.

From these data it could be concluded that the compound with the lowest k'-value was the corresponding β-anomer. To the compound with the highest k'-value the α-anomeric configuration could be assigned. The H-1'(α) and H-1'(β) assignment was done with the aid of the syn-up-field rule. The ^{1}H-nmr spectrum of compound (12α) was recorded in CD_3OD. Since in pure CD_3OD the anomeric proton H_1'(β) of compound (12β) was masked by the OH-signal (4.6-5.0 ppm), its ^{1}H-nmr spectrum was taken in CD_3OD/DMSO-d_6 (50/50) (FIG. 2). Recording of the ^{1}H-nmr spectrum of (12β) in pure DMSO-d_6 gave a very complex picture due to the presence of additional OH- and NH_2-resonance signals.

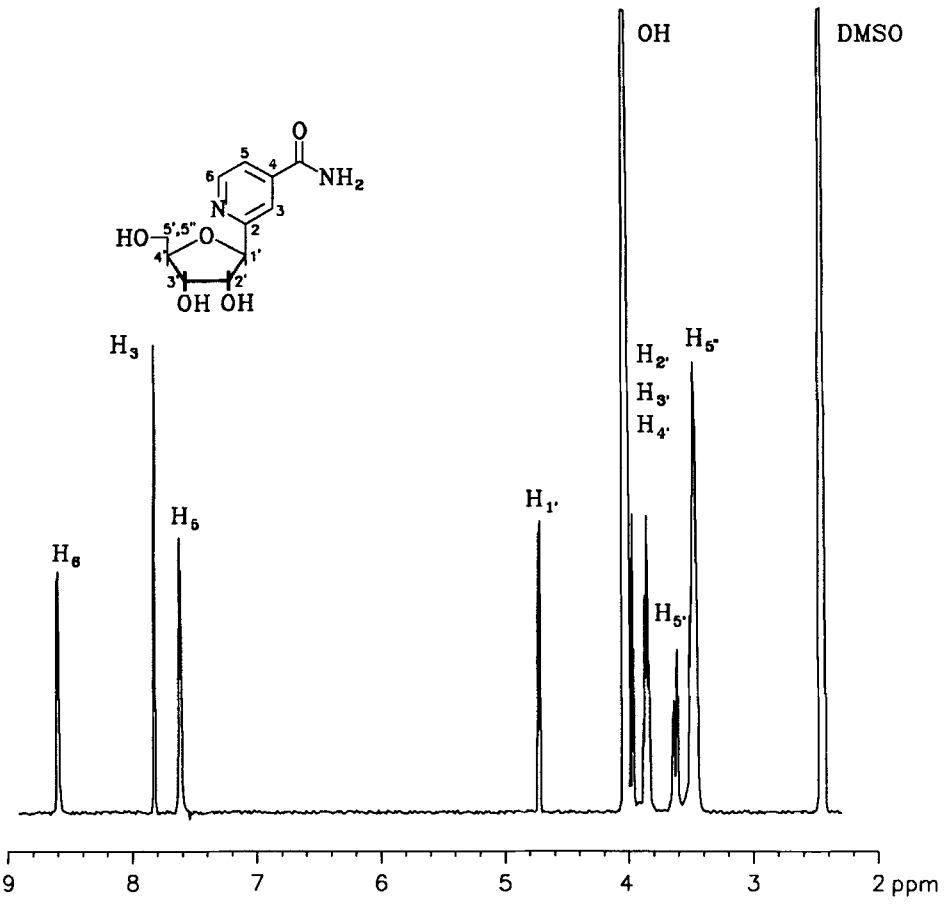


FIG. 2 : 500 MHz ^{1}H-nmr spectrum of (12β) in CD_3OD/DMSO-d_6

BIOLOGICAL STUDIES : In vitro evaluation of the antiviral, cytotoxic and cytostatic activity of compound (12β). (TABLE 2)

The antiviral activity of (12β) was evaluated in a variety of assay systems. Under the conditions where the reference compounds tubercidin, (S)-9-(2,3-dihydroxypropyl)adenine, ribavirin and carbocyclic 3-deazaadenosine showed the usual anti-viral activity, no appreciable antiviral effect was noted for compound 12β (TABLE 2).

Its minimum virus-inhibitory concentration was invariably greater than 400 μg/ml, except for human immunodeficiency virus type 1 (HIV-1), where, because of toxicity of 12β for the host cells, its antiviral activity could not be assessed at a concentration >22 μg/ml.

TABLE 2 : Minimum virus-inhibitory concentration (μg/ml)*

	(12β)	Tubercidin	(S)-DHPA+Ribavirin		C-c^{3}Ado#
HIV-1/MT4 cells	> 22(34)**	-	-	-	-
VSV/HELA	>400	0.07(1.0)	4	20	2
Coxsackie B4/HELA	>400	0.2(1.0)	>400	70	>400
Polio-1/HELA	>400	0.2(1.0)	>400	70	>400
Parainfluenza-3/VERO	>400	>0.1(0.4)	100	70	2
Reo 1/VERO	>400	0.07(0.4)	7	20	0.7
Sindbis/VERO	>400	>0.1(0.4)	>400	70	200
Coxsackie B4/VERO	>400	0.07(0.4)	40	70	7
Semliki forest/VERO	>400	>0.1(0.4)	>400	150	>400
HSV-1/PRK	>400	>0.1(0.4)	>400	>400	200
HSV-2/PRK	>400	>0.1(0.4)	>400	>400	>400
Vaccinia/PRK	>400	0.07(0.4)	40	20	2
VSV/PRK	>400	0.02(0.4)	20	400	2

*Dose required to reduce virus induced cytopathogenicity by 50%.

+(S)-9-(2,3-dihydroxypropyl)adenine.

#Carbocyclic 3-deazaadenosine.

**Values in parentheses refer to the minimum cytotoxic concentration, required to cause a microscopically visible alteration of normal cell morphology (HELA, VERO, PRK) or to reduce the number of viable cells by 50% (MT-4 cells, as assessed by the trypan blue exclusion method).

For detailed information on the methods used to measure the inhibitory effects of the compounds on virus-induced cytopathogenicity in: PRK cells[9]; VERO and HELA cells[10] and MT-4 cells.[11]

The cytostatic activity of 12β was evaluated in a number of tumor cell systems, as described previously[12] : it had a modest inhibitory effect on the proliferation of murine leukemia (L1210), human B-lymphoblast (RAJI), human T-lymphoblast (MOLT/4F) and human T-lymphocyte (MT-4) cells: its 50% inhibitory dose for the growth of these cells was 215, 112, 76 and 35 μg/ml, respectively. Thus, 4-carbamoyl-2-β-D-ribofuranosylpyridine can be considered as a weakly active cytostatic agent.

EXPERIMENTAL

Physical characterization of compounds. ^{1}H-Nmr spectra were obtained using a Bruker WH-360-(360 MHz, R.U.Ghent, Ghent, Belgium), a Bruker-500 (500 MHz, R.U.Ghent, Ghent, Belgium) for compounds (12α) and (12β) or a Jeol JNM-FX-100 (100 MHz, RUCA, University of Antwerp, Antwerpen, Belgium). ^{13}C-Nmr spectra were recorded on a Jeol-JNM-FX-100 (25 MHz, RUCA, University of Antwerp, Antwerpen, Belgium). All nmr spectra were recorded in $CDCl_3$ (TMS as internal reference), except for compound (3) (DMSO-d_6), compound (12α) (CD_3OD) and compound (12β) (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. Hplc analyses were carried out on a Varian Vista 5000 apparatus (columns : Lichrosorb 10 RP 8 (25 cm x 4.6 mm) and Lichrosorb 10 DIOL (25 cm x 4.6 mm ID)). Semipreparative purification of the compounds was done on a Hewlett-Packard HP-1084-B hplc apparatus (columns : Lichrosorb 10 RP 8 (25 cm x 9 mm ID) or Lichrosorb 10 DIOL (25 cm x 8 mm ID)). The compounds were detected by uv (λ_{max}=275 nm).

Syntheses. 2-Amino-4-methylpyridine (1) and D-ribose were purchased from Janssen Chimica (Beerse, Belgium). Silica gel (silica gel 60 PF-254; binder : $CaSO_4$ and Kiesel gel 60, particle size 0.040-0.063 mm (230-400 mesh ASTM)) was purchased from Merck (Belgolabo, Overijse, Belgium). Except where noted, separations on silica gel were performed by centrifugal circular thin layer chromatography (CHROMATOTRON)^R.

2-Bromo-4-methylpyridine (2). A beaker of 1000 ml, containing 70 ml 48% HBr was cooled to -5°C in an ice/NaCl bath. Then 22 g (0.11 mol) of 2-amino-4-methylpyridine (1) was slowly added, keeping the reaction mixture at a constant temperature of 0°C. While stirring vigorously, 17 ml (0.2125 mol) of Br_2 was added. This resulted in the formation of a dark red precipitate, which was treated with a solution of 21 g (0.19 mol) of $NaNO_2$. The temperature was kept at 0°C. Stirring was continued for another hour. When the evolution of N_2 gas ceased, a solution of 45 g (1.13 mol) of NaOH in 115 ml of H_2O was poured into the reaction mixture. The reaction mixture was extracted with diethyl ether (3 x 250 ml). The combined ether layers were dried on magnesium sulfate evaporated and the crude 2-bromo-4-methylpyridine (2) was purified by vacuum distillation (yield 15.8 g (83%)). bp : 100°C (4 mm Hg). ^{1}H-Nmr ($CDCl_3$) δ 8.0 (d, J=5.22 Hz, 1 H, H-6), 7.1 (s, 1 H, H-

3), 6.9 (d, J=5.22 Hz, 1 H, H-5), 2.3 (s, 3 H, CH_3). ^{13}C-Nmr ($CDCl_3$) δ 150.2 (C-4), 149.6 (C-6), 142.2 (C-2), 128.5 (C-3), 123.8 (C-5), 20.6 (CH_3). DCI-mass spectrometry (NH_3) : m/z=189 ($[M+NH_4]^+$,(^{79}Br), 23.7), m/z=172 ($[MH]^+$, (^{79}Br), 100).

2-Bromopyridine-4-carboxylic acid (3). In a two-necked flask of 1000 ml, equipped with a reflux condenser, 5 g (0.029 mol) of 2-bromo-4-methylpyridine (2) and 11.4 g (0.072 mol) of $KMnO_4$ in 125 ml of water were mixed. The reaction mixture was allowed to reflux for 5 h. Then an additional amount of 0.64 g (0.004 mol) of $KMnO_4$ was added. Refluxing was continued for another hour and the reaction mixture was allowed to cool to room temperature. The unreacted 2-bromo-4-methylpyridine (2) was removed by steam distillation and the remaining reaction mixture was filtered. The filtrate was reduced to 100 ml on a rotatory evaporator and acidified with 36 N HCl. The crude 2-bromopyridine-4-carboxylic acid (3) precipitated and was recrystallized from water (yield 1.88 g (32%)). mp : 235°C. ^{1}H-Nmr (DMSO-d_6) δ 8.6 (d, J=5.23 Hz,1 H, H-6), 8.0 (s, 1 H, H-3), 7.8 (d, J=5.23 Hz, 1 H, H-5). ^{13}C-Nmr (DMSO-d_6 δ 164.7 (COOH),151.4 (C-6), 142.0 (C-2), 141.7 (C-4), 127.2 (C-3), 122.5 (C-5). DCI-mass spectrometry (NH_3) : m/z=219 ($[M+NH_4]^+$ (^{79}Br), 24.2), m/z=202 ($[MH]^+$, (^{79}Br), 100), m/z=158 ($[MH]^+$-CO_2, (^{79}Br), 22.1).

Ethyl 2-bromopyridine-4-carboxylate (4). A flask of 250 ml equipped with a reflux condenser and a Dean-Stark apparatus was filled with 5 g (0.022 mol) of 2-bromopyridine-4-carboxylic acid (3), 40 ml of dry benzene (dried over Na-wire), 20 ml of ethanol and 1 ml of 98% H_2SO_4. The contents were allowed to reflux. After 24 h, the reaction mixture was poured into a saturated $NaHCO_3$ solution (250 ml). This solution was extracted with chloroform (3 x 50 ml). The combined organic layers were dried on magnesium sulfate and filtered off. The chloroform was removed under vacuum, yielding 4.6 g (80%) of crude ethyl 2-bromopyridine-4-carboxylate. This compound was immediately used, without any further purification, for the preparation of 2-bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine (6). ^{1}H-Nmr ($CDCl_3$) δ 8.3 (d, J=5.22 Hz, 1 H, H-6), 7.8 (s, 1 H, H-3), 7.6 (d, J=5.22 Hz, 1 H, H-5), 4.3 (q, J=7.46 Hz, 2 H, CH_2), 1.4 (t, J=7.46 Hz, 3 H, CH_3). ^{13}C-Nmr ($CDCl_3$) δ 163.6 (C=O), 150.9 (C-6), 142.8 (C-2), 140.2 (C-4), 127.7 (C-3), 122.0 (C-5), 62.3 (CH_2), 14.2 (CH_3). DCI-mass spectrometry (NH_3) : m/z=247 ($[M+NH_4]^+$, (^{79}Br), 16.4), m/z=230 ($[MH]^+$, (^{79}Br), 100).

2-Bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine (6). In a flask of 50 ml, equipped with a reflux condenser, 5 g (0.021 mol) of ethyl 2-bromopyridine-4-carboxylate (4)

and 2.48 g (0.0273 mol) of 2-amino-2-methylpropanol were refluxed. After 90 min the reaction mixture was allowed to cool to room temperature and the excess of 2-amino-2-methylpropanol was removed by distillation under vacuum. Then 15 ml of 98% H_2SO_4 was added. The solution was stirred and warmed up to 110°C. After 10 min the reaction mixture was cooled and poured into 25% NH_4OH. This solution was extracted with diethyl ether (3 x 50 ml). The combined organic layers were dried on magnesium sulfate, filtered off and evaporated under vacuum. The crude reaction product was purified by vacuum distillation. 2-Bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine (6) crystallized spontaneously (yield : 1.70 g (30%)). bp : 101°C (2 mm Hg). ^{1}H-Nmr ($CDCl_3$) δ 8.3 (d, J=5.60 Hz, 1 H, H-6), 7.9 (s, 1 H, H-3), 7.6 (d, J=5.60 Hz, 1 H, H-5), 4.1 (s, 2 H, CH_2), 1.4 (s, 6 H, 2xCH_3). ^{13}C-Nmr ($CDCl_3$) δ 159.1 (-C=N), 150.4 (C-6), 142.6 (C-2), 138.1 (C-4), 126.7 (C-3), 121.0 (C-5), 79.6 (CH_2), 68.3 (=C=), 28.2 (CH_3). DCI-mass spectrometry (NH_3) : m/z=255 ($[MH]^+$, (^{79}Br), 100).

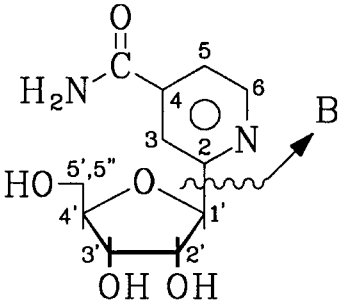
D-Allo/D-altro 2-(2,4;3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine (9). In a three-necked flask of 250 ml, equipped with a $CaCl_2$-tube, a dropping funnel and a gas inlet tube, 0.51 g (2 mmol) of 2-bromo-4-(4,4-dimethyloxazolin-2-yl)pyridine was dissolved in 40 ml of dry 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 30 ml of dry THF containing 0.654 g (2 mmol) of 2,4;3,5-di-O-benzylidene-aldehydo-D-ribose 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 ethyl acetate (3 x 50 ml) and the combined organic layers were dried on magnesium sulfate and filtered off. After evaporation of the ethyl acetate a brown foam was obtained, which was purified by centrifugal circular thin layer chromatography on a Chromatotron^R (silica gel 60 PF-254; binder : $CaSO_4$; eluant : hexane/ethyl acetate (70/30), flow-rate=1 ml/min) and compound (9) was collected (R_F=0.4, yield : 805 mg (80%)). ^{1}H-Nmr ($CDCl_3$) δ 8.6 (dd, J=5.97 Hz, 1 H, H-6), 8.0 (s, 1 H, H-3), 7.6 (d, J=5.97 Hz, 1 H, H-5), 6.9-7.5 (m, 10 H, arom. protons), 5.7 (d, J=7.46 Hz, 1 H, H-1'), 5.5 (s, 2 H, H-6' and H-6''), 4.1 (s, 2 H, CH_2), 3.6-4.4 (m, 5 H, H-2', H-3', H-4', H-5', H-5''), 1.3 (s, 3 H, CH_3). All signals mentioned are split in two due to the presence of the D-allo and D-altro isomers. ^{13}C-Nmr ($CDCl_3$) δ 160.2/160.4 (-C=N), 148.5/148.8 (C-6), 136.0/136.1-137.0/137.2 (C-2 and C-4), 125.7-129.0 (arom. C-atoms), 119.5/119.8-120.6/120.9 (C-3 and C-5), 101.03-

101.7/101.9 (C-6', C-6''), 80.7/81.3 (C-2'), 79.4 (CH_2), 70.7-74.1 (C-1', C-3', C-4'), 68.8 (-C=N), 67.9/68.0 (C-5'), 28.2 (CH_3). DCI-mass spectrometry (NH_3) : m/z=503 ($[MH]^+$, 100).

D-Allo/D-altro 2-(1-O-mesyl-2,4;3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine (10). A 100 ml flask was filled with a solution of 100.6 mg (0.2 mmol) of coupling product (9) in 25 ml of dry pyridine (freshly distilled from CaH_2) and an excess of methanesulphonyl 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 ethyl acetate (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 : CH_2Cl_2/CH_3OH (97/3), flow-rate=1 ml/min). After evaporation of the solvent, the mesylate (10) was isolated as a yellow foam (R_F=0.3, yield : 96.5 mg (83%)). ^{1}H-Nmr ($CDCl_3$) δ 8.7 (d, J=5.67 Hz, 1 H, H-6), 8.1 (s, 1 H, H-3), 7.8 (d, J=5.67 Hz, 1 H, H-5), 7.24-7.7 (m, 10 H, arom. protons), 6.1 (d, J-value could not be determined, 1 H, H-1'), 5.67 (s, 2 H, H-6' and H-6''), 4.2 (s, 2 H, CH_2), 3.9-4.6 (m, 5 H, H-2', H-3', H-4', H-5', H-5''), 3.0 (s, 3 H, CH_3OSO_2-), 1.4 (s, 3 H, CH_3). All signals mentioned are split in two due to the presence of D-allo and D-altro isomers. ^{13}C-Nmr ($CDCl_3$) δ 158.9/154.9 (C=N), 148.5/148.0 (C-6), 135.9/135.7-135.5/135.4 (C-2 and C-4), 124.7-128.2 (arom. C-atoms), 120.9/120.3-120.0/118.9 (C-3 and C-5), 100.6/100.3 (C-6' and C-6''), 81.1/78.8 (C-1'), 78.8/77.7 (C-2'), 78.3 (CH_2), 67.5 (C-5'), 67.1 (=C=), 37.6/37.5 (CH_3OSO_2), 27.2 (CH_3). DCI-mass spectrometry (NH_3) : m/z=581 ($[MH]^+$, 100).

4-Carbamoyl-2-D-ribofuranosylpyridines (12α and 12β). In a flask of 100 ml, 100 mg (0.17 mmol) of D-allo/D-altro 2-(1-O-mesyl-2,4;3,5-di-O-benzylidenepentitol-1-yl)-4-(4,4-dimethyloxazolin-2-yl)pyridine (10) was dissolved under stirring in 20 ml of CF_3COOH. Then 5 ml of water was added and the reaction mixture was allowed to stand at room temperature. After 15 min the mixture was poured into 200 ml of water and the aqueous layer was washed with chloroform (3 x 100 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, giving the crude product (11). The latter 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 50°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 9 mm ID, eluant : CH_3OH/H_2O (10/90), retention time = 2.81 min, uv-detection, λ_{max}=275 nm). α/β-Anomers were not separated but collected together. α/β-Separation was carried out on a Lichrosorb 10 DIOL column (25 cm x 8 mm ID, eluant : 5% 2-propanol, 95% CH_2Cl_2/CH_3COOH (99.5%-0.5%), flow-rate=6 ml/min, retention time of the α-anomer : 18.45 min, retention time of the β-anomer : 10.13 min, uv-detection, λ_{max}=275 nm), giving 12.24 mg of the β-isomer (12β) and 9.62 mg of the α-isomer (12α), both as a colorless syrup. ^{1}H-Nmr δ-values : see TABLE 1. ^{13}C-Nmr (CD_3OD) δ 170.1 (C=O (β)), 169.9 (C=O (α)), 163.6 (C-2 (α)), 162.2 (C-2 (β)), 150.6 (C-6 (β)), 150.1 (C-6 (α)), 143.8 (C-4 (β)), 143.4 C-4 (α)), 122.0-122.3 (C-3 (α,β), C-5 (α,β)), 84.5-86.5 (C-1' (α,β), C-4' (α,β)), 78.4 (C-2' (β)), 72.9 (C-2' (α)), 72.8 (C-3' (α)), 72.6 (C-3' (β)), 63.4 (C-5' (α,β)). DCI-mass spectrometry (NH_3) : m/z=255 ([MH^+], 100%), m/z=165 ([B+44]$^+$, 8.2%), m/z=151 ([B+30]$^+$, 7.3%) (B=heterocyclic moiety). Anal. calc. for $C_{11}H_{14}N_2O_5$: C, 51.96%; H, 5.55%; N, 11.02%. Found : C, 51.75%; H, 5.61%; N, 10.96%.



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