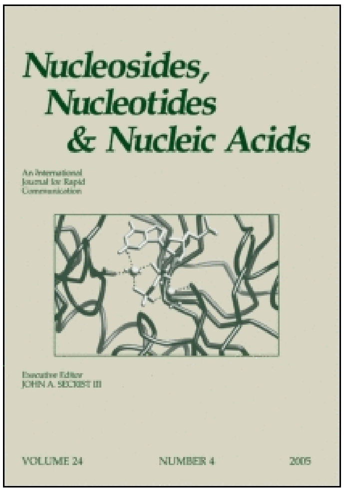

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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME D-XYLOFURANOSYL-PYRIDINE C-NUCLEOSIDES

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ABSTRACT.

The addition reaction of either 3-bromo-5-lithiopyridine (2a) or 3-cyano-5-lithiopyridine (2b) to 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1) gave a D-gulo/D-ido mixture of respectively 3-bromo- and 3-cyano-5-(2,4;3,5-di-O-benzylidene-pentitol-1-yl)pyridine (3a,b). Mesylation of C-1' followed by reaction with CF_3COOH/H_2O resulted in the formation of the corresponding D-xylofuranosyl pyridine C-nucleosides. 3-Cyano-5-D-xylofuranosylpyridine (5b) was converted to 3-carbamoyl-5-D-xylofuranosylpyridine (6) with Amberlite IRA 400 (OH^-). The D-xylofuranosyl C-nucleosides were evaluated for their antiviral and cytostatic activity. No significant activity was found.

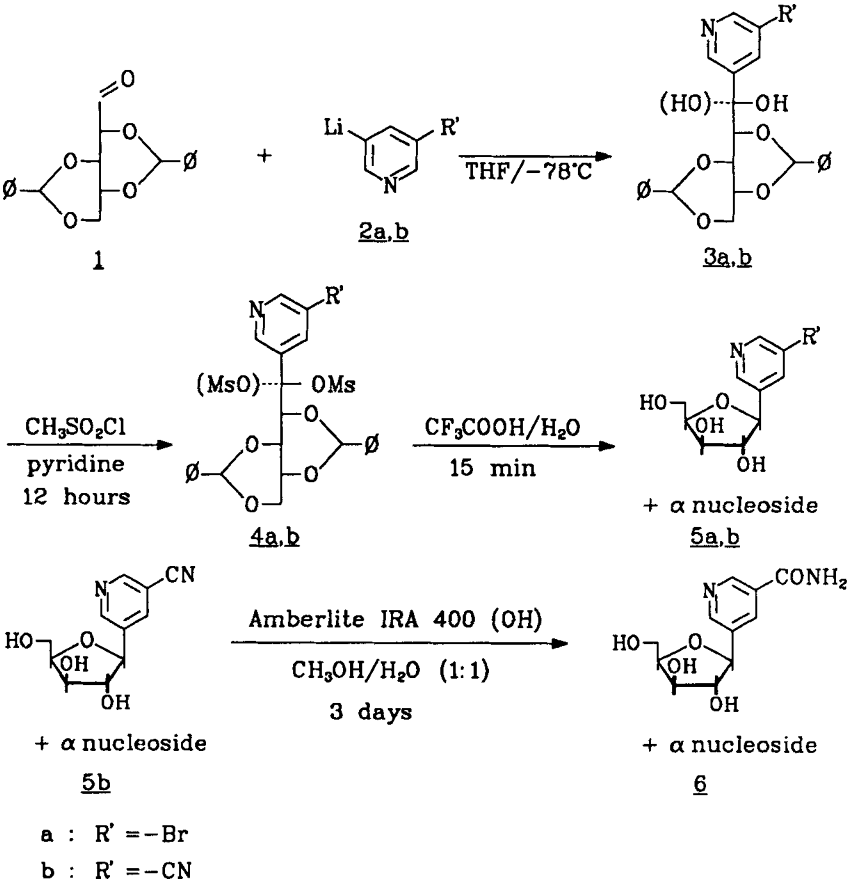
INTRODUCTION.

In the series of D-ribofuranosyl pyridine C-nucleosides, described by us and others, 3-carbamoyl-5-β-D-ribofuranosyl[1]- and 4-carbamoyl-2-β-D-ribofuranosylpyridine[2] showed mild *in vitro* cytostatic activity when evaluated against either P-815, CCRF-CEM, F-MOLT-3 and HL-60 tumor cells or L1210, Raji, Molt/4F and MT-4 cells, respectively.

To study the effect of structural modifications in the sugar moiety on the biological activity, the synthesis of D-xylo-, D-arabino- and D-lyxo pyridine C-nucleosides was undertaken. In this paper, we present the results obtained for some D-xylofuranosyl compounds.

RESULTS AND DISCUSSION.

Pyridine C-nucleosides characterized by the presence of a D-ribofuranosyl moiety can be obtained in an elegant way using lithiopyridines and an appropriate protected sugar derivative. In this approach it is important that pyridine derivatives are selected which can be regioselectively lithiated in high yields and with a structure which allows the introduction of a functional group at a well defined stage of the total synthesis. Several pyridine compounds such as dibromopyridines[3] and bromopyridines with an amide function masked as an oxazoline ring[2], fulfill these requirements and can therefore be considered as candidates for this strategy.



SCHEME 1 (Method 1)

In the past, several of these compounds have been used by us in a reaction with 2,4:3,5-di-O-benzylidene-aldehydo-D-ribose allowing the preparation of D-ribofuranosyl-pyridine C-nucleosides in good yields. Therefore we decided to synthesize the D-xylofuranosyl compounds by using 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose[4,5] in combination with 3,5-dibromopyridine and 3-bromo-5-cyanopyridine as starting materials according to SCHEMES 1 and 2.

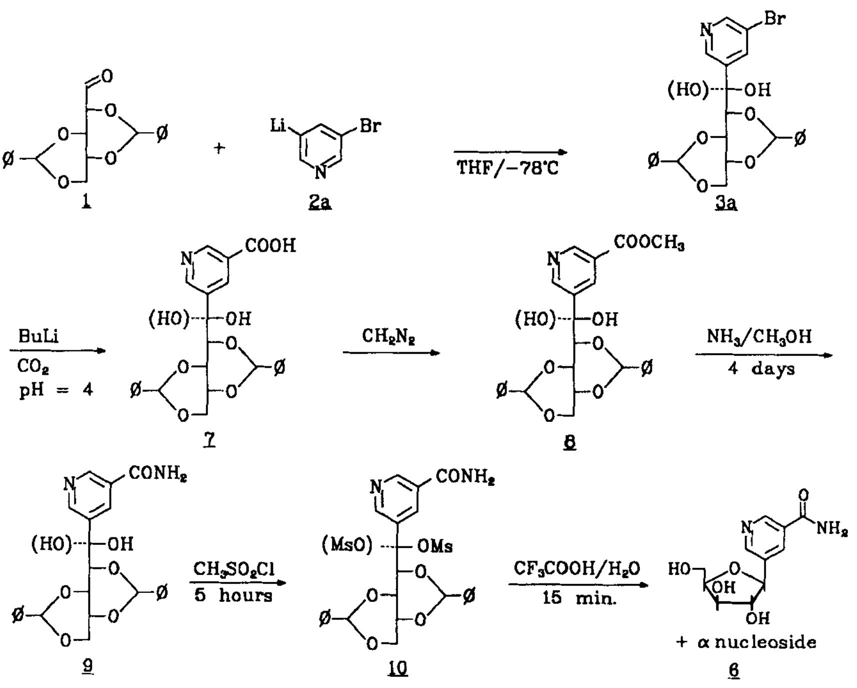
The use of either 3,5-dibromopyridine or 3-bromo-5-cyanopyridine[6] offered two alternative routes to the preparation of 3-carbamoyl-5-D-xylofuranosylpyridine (6) when the respective lithio intermediates were reacted with 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1).

A crucial step in the total synthesis of this kind of C-nucleosides is the *in situ* formation of the lithiopyridines. The lithiation of 3,5-dibromopyridine and 3-bromo-5-cyanopyridine was performed earlier by Watanabe et al[1,6] : 3,5-dibromopyridine[1], dissolved in dry Et_2O, was lithiated by adding 1 eq. of BuLi at -50°C over a period of 10 min. After 15 min. at -50°C, the temperature was lowered to -78°C and the protected sugar derivative was then added. A somewhat similar procedure was used by this author for the lithiation of 3-bromo-5-cyano-pyridine[6], but the halogen-exchange reaction was performed at -78°C.

We found that both 3,5-dibromopyridine and 3-bromo-5-cyanopyridine could be converted to the corresponding lithio derivatives in good yields by performing the reaction in dry THF at -78°C, and by adding 1 eq. of BuLi in a single step. After 4 min. at -78°C, a solution of 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1) was added dropwise giving the corresponding D-gulo/D-ido compounds (3a,b).

This procedure is easier to perform and the D-gulo/D-ido adducts (3a,b) were isolated in good yields[7].

Treatment of these compounds with methanesulphonyl chloride in dry pyridine for 12 hours, resulted in the formation of the mesylates (4a,b), which were converted to the corresponding nucleosides (5a,b) using a mixture of CF_3COOH/H_2O (4:1). The α/β ratio was 35/65 as determined by HPLC on the crude nucleoside mixture. Purification and α/β separation was performed on a High Coverage RSil C8 10



SCHEME 2 (Method 2)

micron (25cm x 10mm I.D.) column using a CH_3OH/H_2O mixture as mobile phase or by column chromatography on silica gel.

An α/β-mixture of 3-cyano-5-D-xylofuranosylpyridine (5b) was dissolved in a mixture of CH_3OH/H_2O (1:1) and stirred for 3 days in the presence of Amberlite IRA-400 (OH^-)[6] to yield an α/β mixture of 3-carbamoyl-5-D-xylofuranosylpyridine (6).

The latter nucleoside was also synthesized by using an alternative route[8], as depicted in SCHEME 2. The addition reaction of 3-bromo-5-lithiopyridine (2a) with 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1) yielded a mixture of D-gulo/D-ido addition products (3a), which were not isolated but lithiated for a second time by adding another 2.1 eq. of BuLi at -78°C. After 3 min. the reaction was quenched by pouring the contents on a large excess of freshly prepared dry ice. After adjusting the pH to 4 (citrate buffer) compound (7) was isolated by extraction and without further purification converted to D-gulo/D-ido

3-methoxycarbonyl-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (8). The methyl ester (8) was suspended in a solution of saturated methanolic ammonia and stirred for 4 days. After evaporation of the solvent, the D-gulo/D-ido 3-carbamoyl-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (9) was obtained, which was mesylated with CH_3SO_2Cl in dry pyridine. The mesylation reaction was quenched after 5 hours by pouring the reaction mixture in a saturated $NaHCO_3$-solution. Application of longer reaction times must be avoided since a slow conversion of the carbamoyl to a cyano group has been noticed under these conditions[3]. The D-gulo/D-ido 3-carbamoyl-5-(1-O-mesyl-2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (10) was treated with CF_3COOH/H_2O (4:1) at room temperature in order to obtain the crude 3-carbamoyl-5-D-xylofuranosyl-pyridine (6), which was purified and α/β-separated on a RoSil C8 5 micron column (25cm x 4.6mm I.D.) using H_2O as mobile phase.

Comparison of methods 1 and 2 showed a difference of only 7% in overall yield for compound (6) (method 1 : 49%, method 2 : 42%). However from an experimental point of view, method 1 was found to be more reproducable since in this approach the carboxylation procedure was absent. The latter reaction is highly sensitive to moisture so that the presence of even small amounts of H_2O will decrease the yield of (7) drastically.

Because the cyano function in (5b) can be transformed to other functional groups then carbamoyl (thiocarbamoyl[9], formyl[10] etc.), method 1 offers an easy route to other functionalized C-nucleosides.

An alternative i.e. the introduction of functional groups after direct lithiation of (5a) was not investigated since, according to literature data[11], polylithiated nucleosides react with electrophyles in low yields due to the insolubility of the former compounds.

In view of the aforesaid, method 1 should be preferred to method 2.

Structure identification by NMR.

Structure identification and α/β-assignment was done by 360 MHz ^1H-NMR spectroscopy (compounds (6α and 6β)), 500 MHz ^1H-NMR spectroscopy (compounds

TABLE 1 : ^{1}H-NMR data of (5a), (5b) and (6) : δ-values in ppm.

	5aα	5aβ	5bα	5bβ	6α	6β
H-2	8.511(d)	8.582(d)	8.777(d)	8.863(d)	8.67(d)	8.68(d)
H-4	8.025(t)	7.905(t)	8.162(t)	8.283(t)	8.28(t)	8.16(t)
H-6	8.471(d)	8.491(d)	8.764(d)	8.788(d)	8.89(d)	8.94(d)
H-1'	5.239(d)	3.988(d)	5.304(d)	4.780(d)	5.32(d)	4.06(d)
H-2'	4.162(dd)	3.58 - 3.73	4.194(dd)	3.972(dd)	4.20(dd)	3.60 - 3.76
H-3'	4.266(dd)		4.274(dd)	4.161(dd)	4.29(dd)	
H-4'	4.373(m)		4.393(m)	4.224(m)	4.42(m)	
H-5'	3.850(dd)		3.858(dd)	3.930(dd)	3.88(dd)	
H-5''	3.820(dd)		3.838(dd)	3.890(dd)	3.84(dd)	

(5aα, 5aβ, 5bα and 5bβ) and 25 MHz ^{13}C-NMR spectroscopy.

The spectra were recorded in CD$_3$OD-solutions, using the residual solvent signal as internal reference, and are summarized in TABLES 1, 2 and 3.

The assignment of the anomeric configuration in D-xylofuranosyl C-nucleosides (5a), (5b) and (6) was achieved by using several criteria.

A method which is generally used for D-ribofuranosyl compounds[12,13] is based upon the syn-upfield rule which states that δH-1’(α) > δH-2’(β). This emperical rule is a result from a different spacial interaction between H-1’ and the hydroxyl function at C-2’ in each anomer. Since the configuration at C-2’ in the D-xylofuranosyl compounds is identical to the configuration at C-2’ in the D-ribofuranosyl compounds, the α/β-assignment for compounds (5a) to (6) was done by this rule.

As reported by E. De Vos et al[14] for a series of D-ribofuranosyl C-nucleosides another criterium can be used. In this series of compounds a syn-upfield effect was noted for H-2’(β). Probably this phenomenon originates from interactions of H-2’ with the pyridine moiety. This regularity was also observed in the series of

TABLE 2 : ^{1}H-NMR data of (5a), (5b) and (6) : coupling constants in Hz.

	5aα	5aβ	5bα	5bβ	6α	6β
J(2,4)	2.2	2.2	2.0	2.0	1.6	1.7
J(4,6)	1.4	1.7	2.1	1.9	1.5	1.6
J(1',2')	3.3	1.9	3.3	3.8	3.3	2.0
J(2',3')	1.3	a	1.3	2.5	1.0	a
J(3',4')	3.4	a	3.4	4.3	2.6	a
J(4',5')	5.1	a	5.0	4.5	5.1	a
J(4',5'')	6.4	a	6.5	6.0	6.7	a
J(5',5'')	-11.6	a	-11.6	-11.8	-11.6	a

a: could not be determined.

TABLE 3 : ^{13}C-NMR data of (5a), (5b) and (6) : δ-values in ppm.

	5aα	5aβ	5bα	5bβ	6α	6β
C-2	149.8	151.0	152.9	152.1	151.8	150.9
C-3	121.4	122.0	110.8	111.0	130.5	131.2
C-4	139.8	137.6	140.1	139.4	136.2	134.3
C-5	138.5	137.3	137.2	138.9	136.4	135.3
C-6	147.6	146.8	151.7	151.9	148.1	149.1
C-1'	81.3	72.8	81.3	84.8	81.7	73.9
C-2'	80.0	64.9	79.9	83.5	80.0	64.8
C-3'	79.7	63.9	78.9	79.2	78.9	63.9
C-4'	83.2	74.3	83.3	85.4	83.1	74.4
C-5'	62.0	53.9	61.9	61.9	62.0	54.4
-CN	-	-	117.7	117.5	-	-
-CONH$_2$	-	-	-	-	169.9	169.6

D-xylofuranosyl C-nucleosides. This was not surprising since a more or less analogous interaction can be expected between H-2' and the heterocyclic base. As can be seen in TABLE 1, the H-2' proton from (5bβ) resonates upfield with respect to H-2' of (5bα). In compounds (5aβ) and (6β) it was not possible to determine the exact value of H-2'. However, since all protons, except H-1', resonate upfield with respect to the same protons in the corresponding α-anomer it is obvious that also for these compounds δH-2'(α) > δH-2'(β).

A NOE-DIFF[15] spectrum (500 MHz) of compound (5bα) confirms the α/β-assignment, based on these syn-upfield rules. Indeed, by saturating H-1', no NOE factors at H-3' and H-4' could be observed, only H-2' had a NOE factor of ± 9%. This NOE factor agrees very well with NOE factors for H-2' for N-α-ribofuranosides[15,16] (10.0 - 11.3), when irradiating H-1'. The NOE factor for the β-anomers lies between 1.6 and 2.2%. To our knowledge, no reference NOE data for C-ribofuranosides or xylofuranosides (either N- or C-nucleosides) are available.

The assignment of the carbon atoms of nucleosides (5aα) and (5bα) was accomplished using Hetcor spectra. The sequence of the sugar carbon atoms, obtained from these spectra, is C-4', C-1', C-2', C-3' and C-5' when going upfield. This sequence was used to assign the carbon atoms of the other nucleosides. The assignment of C-5' for all nucleosides was checked, using DEPT-spectra, in which CH_2-signals appear with negative intensity.

BIOLOGICAL STUDIES.

Nucleosides (5aα), (5aβ) and (5b) were evaluated for their inhibitory effect on the replication of : herpes simplex virus-1 (strain KOS), herpes simplex virus-2 (strain G), vaccinia virus and vesicular stomatitus virus (VSV) in E_6SM cell cultures; VSV, Coxsackie virus B4 and polio virus-1 in HeLa cell cultures; and para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus and Semliki forest virus in Vero cell cultures. The minimum inhibitory concentration (i.e. the compound concentration required to reduce the virus-induced cytopathogenicity by 50%) was higher than 400 μg/ml. The minimum cytotoxic concentration (i.e. the compound concentration required to cause a microscopically detectable alteration of normal cell morphology) was also higher than 400 μg/ml. Neither anti-HIV-1 nor anti-HIV-2-activity or cytotoxicity were observed in MT-4 cells (>200 μg/ml).

Compound (5aα), (5aβ), (5b), (6α) and (6β) showed no cytostatic activity against murine leukemia L1210, human B-lymphoblast Raji or human T-lymphoblast Molt/4F cells at concentrations up to 200 μg/ml.

EXPERIMENTAL.

General methods.

^{1}H-NMR spectra were recorded on a 100 MHz Jeol JNM-PS-100 spectrometer, a Bruker WH-360 NMR spectrometer (R.U.Ghent) or a Bruker-500 (R.U.Ghent). ^{13}C-NMR spectra were recorded on a Jeol FX-100 connected to a TI-980B computer system. The NOE experiments were run on a Bruker-500 (R.U.Ghent) spectrometer, using the NOEDIFF mode of the Bruker software package. The CD_3OD solutions were degassed by bubbling N_2 through it. DCI-mass spectra were run on a Ribermag 10-10B (Nermag SA) quadrupole mass spectrometer, equipped with a Sidar data system. Primary ionization was performed by 70 eV electrons using an emission current of 0.08 mA, the pressure in the ion source was around 0.3 Torr. Separations on silica gel were performed by preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron® (Kieselgel 60 PF254 gipshaltig, purchased from Merck), layer thickness was 2 mm.

Semi-preparative purifications and α/β-separations were accomplished on a Hewlett-Packard HP-1084-B-HPLC apparatus.

Elemental analyses were recorded at Janssen Pharmaceutica (Beerse, Belgium).

Reactions involving organometallic reagents were performed in oven-dried glassware under a dry N_2 atmosphere. THF was distilled from $LiAlH_4$, and pyridine from CaH_2, prior to use.

D-xylose and BuLi (1.6 M in hexane) were purchased from Janssen Chimica (Beerse, Belgium), 3,5-dibromopyridine from Lancaster Synthesis Ltd. (Mundolsheim, France). The cytostatic and antiviral assays were performed according to previously published procedures[17-20].

Synthesis.

D-gulo/D-ido 3-bromo-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (3a).

A three necked flask of 100 ml, equipped with a dropping funnel, $CaCl_2$ tube and dry N_2 inlet system, was filled with 3,5-dibromopyridine (436 mg, 1.84 mmol) dissolved in 60 ml dry THF. The solution was cooled in a CO_2/acetone bath to -78°C, and 1.15 ml of BuLi (1.6 M in hexane, 1.84 mmol) was added while stir-ring. After 4 min., a solution of 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1) (500 mg, 1.53 mmol) in 30 ml dry THF was added over a period of 10 min. After 2 hours at -78°C, the solution was allowed to warm up, overnight, to room temperature.

The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (200 ml) and H_2O (200 ml). The layers were separated, and the aqueous layer was extracted again with EtOAc (2x100 ml). The combined organic phases were washed with saturated brine (2x50 ml), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give D-gulo/D-ido 3-bromo-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (3a) (641 mg, 86%) as a light brown solid.

^{1}H-NMR (DMSO-d_6) : δ 8.6 (1H, d, J=2.2 Hz, H-2), 8.5 (1H, d, J=1.6 Hz, H-6), 8.1 (1H, t, H-4), 7.2 - 7.6 (10H, m, aromatic protons), 6.0 (1H, d, J=4.9 Hz, H-1'), 5.75 (1H, s, H-6'), 5.6 (1H, s, H-6"), 4.0 - 4.8 (5H, m, H-2', H-3', H-4', H-5', H-5"). All signals mentioned are present in pairs due to the presence of the D-gulo and D-ido isomers.

^{13}C-NMR (DMSO-d_6) : δ 148.2/148.8 (C-2), 146.4/147.7 (C-6), 140.5/140.7 (C-4), 137.2/137.4 (C-5), 138.1 - 138.5 and 125.7 - 128.5 (aromatic C-atoms), 119.6/119.9 (C-3), 98.9 - 99.5 (C-6', C-6"), 80.2/80.3 - 66.3 (C-1', C-2', C-3', C-4'), 69.2/69.3 (C-5').

DCI-mass spectrometry (NH_3) : m/z = 484 ($[MH]^+$(^{79}Br), 100%).

D-gulo/D-ido 3-cyano-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (3b).

A three necked flask of 100 ml, equipped with a dropping funnel, $CaCl_2$ tube and dry N_2 inlet system, was filled with 3-bromo-5-cyanopyridine (310 mg, 1.69 mmol) dissolved in 60 ml dry THF. The solution was cooled to -78°C in a CO_2/acetone bath, and 1.05 ml of BuLi (1.6 M in hexane, 1.69 mmol) was added while stirring. After 3 min., a solution of 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1) (500 mg, 1.53 mmol) in 30 ml dry THF was added over a period of 10 min. After 2 hours at -78°C, the solution was allowed to warm up, overnight, to room temperature.

The solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 (100 ml) and H_2O (100 ml). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2x50 ml). The combined CH_2Cl_2 layers were dried over $MgSO_4$. After evaporation of the solvent, a brown solid was obtained, which was purified by preparative CCTLC (CH_2Cl_2/CH_3OH (98:2), flow-rate 5 ml/min.), and (3b) was collected (464 mg, 70%).

^{1}H-NMR (DMSO-d_6) : δ 8.90 (1H, d, J=2.0 Hz, H-2), 8.85 (1H, d, J=2.1 Hz, H-6), 8.30 (1H, t, H-4), 7.2 - 7.6 (10H, m, aromatic protons), 6.1 (1H, d, J=4.9 Hz, H-1’), 5.75 (1H, s, H-6’), 5.6 (1H, s, H-6"), 4.1 - 4.9 (5H, m, H-2’, H-3’, H-4’, H-5’, H-5"). All signals mentioned are present in pairs due to the presence of the D-gulo and D-ido isomers.

^{13}C-NMR (DMSO-d_6) : δ 152.1/152.5 (C-2), 151.0/151.3 (C-6), 138.2/139.0 (C-4), 136.9/138.5 (C-5), 138.0 - 138.3 and 125.7 - 128.6 (aromatic C-atoms), 116.8/116.9 (CN), 108.4/108.6 (C-3), 99.0 - 99.5 (C-6’, C-6"), 80.1/81.0 and 66.4 - 69.8 (C-1’, C-2’, C-3’, C-4’, C-5’).

DCI-mass spectrometry (NH_3) : m/z = 431 ($[MH]^+$, 100%).

D-gulo/D-ido 3-bromo-5-(1-O-mesyl-2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (4a) and D-gulo/D-ido 3-cyano-5-(1-O-mesyl-2,4:3,5-di-O-benzylidene-1-yl)pyridine (4b).

To a solution of the D-gulo/D-ido addition products (3a,b) (1.16 mmol) in dry pyridine (50 ml), CH_3SO_2Cl (2 ml) was added at room temperature. The reaction mixture was stirred for 12 hours and quenched by pouring the mixture in a saturated $NaHCO_3$-solution (300 ml). The aqueous layer was extracted with CH_2Cl_2 (1x100 ml, 2x50 ml) and the combined CH_2Cl_2 layers were dried over $MgSO_4$. Evaporation of the solvent yielded a yellow foam, which was purified by preparative CCTLC (eluant : CH_2Cl_2/CH_3OH (98:2), flow-rate 5 ml/min.). Yields : compound (4a) : 85%, compound (4b) : 90%.

Compound (4a) : ^{1}H-NMR ($CDCl_3$) : δ 8.7 (1H, d, J=2.2 Hz, H-2), 8.6 (1H, d, J=1.9 Hz, H-6), 7.9 (1H, t, H-4), 7.3 - 7.6 (10H, m, aromatic protons), 5.9 (1H, d, J=8.8 Hz, H-1’), 5.7 (1H, s, H-6’), 5.5 (1H, s, H-6"), 3.9 - 4.4 (5H, m, H-2’, H-3’, H-4’, H-5’, H-5"), 2.9 (3H, s, $-OSO_2CH_3$). All signals are present in pairs due to the presence of the D-gulo and D-ido isomers.

^{13}C-NMR ($CDCl_3$) : δ 151.6/152.4 (C-2), 148.5/148.6 (C-6), 138.6/138.9 (C-4), 135.7/135.9 (C-5), 139.3 - 139.9 and 126.8 - 129.8 (aromatic C-atoms), 120.8/121.2

(C-3), 100.9 - 101.2 (C-6', C-6''), 79.3/81.1 and 69.1 - 76.3 (C-1', C-2', C-3', C-4', C-5'), 38.7/38.9 ($-OSO_2CH_3$).

DCI-mass spectrometry (NH_3) : m/z = 562 ($[MH]^+$(^{79}Br), 100%).

Compound (4b) : ^{1}H-NMR ($CDCl_3$) : δ 8.9 (1H, d, J=2.2 Hz, H-2), 8.7 (1H, d, J=2.0 Hz, H-6), 8.0 (1H, t, H-4), 7.3 - 7.6 (10H, m, aromatic protons), 5.9 (1H, d, J=8.5 Hz, H-1'), 5.6 (1H, s, H-6'), 5.5 (1H, s, H-6''), 3.9 - 4.5 (5H, m, H-2', H-3', H-4', H-5', H-5''), 2.9 (3H, s, $-OSO_2CH_3$). All signals mentioned are present in pairs due to the presence of the D-gulo and D-ido isomers.

^{13}C-NMR ($CDCl_3$) : δ 153.8/153.9 (C-2), 153.2/153.5 (C-6), 139.6/140.0 (C-4), 132.4/134.5 (C-5), 138.8 - 139.3 and 127.3 - 129.8 (aromatic C-atoms), 117.0 (CN), 110.4/110.9 (C-3), 101.0 - 101.2 (C-6', C-6''), 79.3/80.7 and 69.1 - 76.2 (C-1', C-2', C-3', C-4', C-5'), 38.7/38.9 ($-OSO_2CH_3$).

DCI-mass spectrometry (NH_3) : m/z = 509 ($[MH]^+$, 100%).

α- and β-3-bromo-5-D-xylofuranosylpyridine (5a) and α- and β-3-cyano-5-D-xylofuranosylpyridine (5b).

The mesylates (4a,b) (0.50 mmol) were dissolved in 25 ml of a mixture of CF_3COOH/H_2O (4:1), and the reaction mixture was stirred for 15 min. at room temperature. The reaction mixture was poured into 200 ml H_2O and washed with CH_2Cl_2 (3x40 ml). After evaporation of the aqueous solution, the residue was redissolved in water, neutralised to pH=7 with NH_3 and again evaporated to dryness, yielding compound (5a) (123 mg, 85%) and compound (5b) (107 mg, 90%).

The α/β-mixture of 3-bromo-5-D-xylofuranosylpyridine (5a) was purified and α/β separated by HPLC on a High Coverage RSil C8 10 micron, 25 cm x 4.6mm I.D. column from Alltech (flow-rate = 5 ml/min., injection volume = 40 μl containing 7 mg product, UV-detection : 270 nm, retention time of the α-anomer : 7.20 min., retention time of the β-anomer : 9.47 min.), using CH_3OH/H_2O (30:70) as mobile phase.

DCI-mass spectrometry (NH_3): m/z = 290 ($[MH]^+$(^{79}Br), 100%), m/z = 200 ($[B+44]^+$ (^{79}Br), 40.7%), m/z = 186 ($[B+30]^+$(^{79}Br), 4.7%), (B = heterocyclic moiety).

Elemental analysis for $C_{10}H_{12}BrNO_4$: calc. C, 41.40%; H, 4.17%; Br, 27.54%; N, 4.83%. Found : C, 41.15%; H, 4.20%; Br, 27.37%; N, 4.80%.

The α/β-mixture of 3-cyano-5-D-xylofuranosylpyridine (5b) was purified and α/β-separated by column chromatography (30cm x 3cm I.D.) on silica gel (Kieselgel 60, particle size 0.040 - 0.063 mm (230 - 240 mesh ASTM), purchased from Merck, eluting

with CH_2Cl_2/CH_3OH (85:15), R_f value α-anomer : 0.35, R_f value β-anomer : 0.40).
DCI-mass spectrometry (NH_3) : m/z = 254 ($[MNH_4]^+$, 5.5%), m/z = 237 ($[MH]^+$, 100%), m/z = 147 ($[B+44]^+$, 1.9%), (B = heterocyclic moiety).
Elemental analysis for $C_{11}H_{12}N_2O_4$: calc. C, 55.93%; H, 5.12%; N, 11.86%. Found : C, 55.96%; H, 5.14%; N, 11.80%.

α- and β- 3-carbamoyl-5-D-xylofuranosylpyridine (6).

a) An α/β-mixture of 3-cyano-5-D-xylofuranosylpyridine (5b) (200 mg, 0.85 mmol) was dissolved in a mixture of CH_3OH/H_2O (1:1) (20 ml) and stirred for 3 days in the presence of Amberlite IRA-400 (OH^-) (800 mg). The resin was filtered and washed with CH_3OH (4x10 ml). The combined filtrate and washings were concentrated in vacuo to give the crude α- and β- 3-carbamoyl-5-D-xylofuranosyl-pyridine (6) (185 mg, 86%). Purification and α/β-separation was performed by HPLC on a RoSil C8 5 micron, 25cm x 4.6mm I.D. column from Alltech (flow-rate = 1.5 ml/min., injection volume = 30 μl containing 2 mg product, UV-detection : 270 nm, retention time of the α-anomer : 7.29 min., retention time of the β-anomer : 10.53 min), using H_2O as mobile phase.

b) The mesylate (9) (500 mg, 0.95 mmol) was dissolved in a mixture of CF_3COOH/H_2O (4:1) and the mixture was stirred for 15 min. at room temperature. The reaction mixture was poured into 200 ml H_2O and washed with CH_2Cl_2 (3x40 ml). After evaporation of the aqueous solution, the residue was redissolved in H_2O, neutralised with NH_3 and again evaporated to dryness. The resulting sirup (212 mg, 88%) was purified and α/β-separated as mentioned earlier.
DCI-mass spectrometry (NH_3) : m/z = 255 ($[MH]^+$, 100%), m/z = 165 ($[B+44]^+$, 12.8%), m/z = 151 ($[B+30]^+$, 26.0%), (B = heterocyclic moiety).
Elemental analysis for $C_{11}H_{14}N_2O_5$: calc. C, 51.97%; H, 5.55%; N, 11.02%. Found : C, 51.86%; H, 5.58%; N, 10.95%.

D-gulo/D-ido 3-methoxycarbonyl-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (8).

A three necked flask of 100 ml, equipped with a dropping funnel, $CaCl_2$ tube and dry N_2 inlet system, was filled with 3,5-dibromopyridine (436 mg, 1.84 mmol) dissolved in 60 ml dry THF. The solution was cooled in a CO_2/acetone bath to -78°C, and 1.15 ml BuLi (1.6M in hexane, 1.84 mmol) was added while stirring. After 4 minutes, a solution of 2,4:3,5-di-O-benzylidene-aldehydo-D-xylose (1) (500 mg, 1.53 mmol) in 30 ml dry THF was added over a period of 10 min. After 2 hours at -78°C, the solution was allowed to warm up, overnight, to room temperature. The so formed D-gulo/D-ido 3-bromo-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (3a) was not isolated. Instead, the reaction mixture was again cooled to -78°C. Then 2 ml BuLi (3.21 mmol) was added, and after a reaction time of 3 min., the contents were poured on a large excess of dry ice (200 g). After evaporation of the CO_2, the reaction mixture was evaporated in vacuo to dryness. The residue was dissolved in H_2O and washed with CH_2Cl_2 (3x50 ml). The aqueous layer was acidified to pH=4 (citrate buffer) and extracted with EtOAc. The organic layer was washed with brine (3x30 ml), dried over $MgSO_4$, and concentrated in vacuo to obtain a lightbrown solid.

The carboxylic acid (7) (600 mg, 1.33 mmol) was dissolved in THF, cooled to 0°C and an excess (2.5 eq.) of ethereal diazomethane was added. After evaporation of the solvent, the residue was recristallized in CH_3OH, giving pure D-gulo/D-ido 3-methoxycarbonyl-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)-pyridine (8) (478 mg, 67%).

^{1}H-NMR (DMSO-d_6) : δ 9.1 (1H, d, J=1.7 Hz, H-6), 8.85 (1H, d, J=2.4 Hz, H-2), 8.45 (1H, t, H-4), 7.3 - 7.7 (10H, m, aromatic protons), 6.1 (1H, d, J=5 Hz, H-1'), 5.85 (1H, s, H-6'), 5.7 (1H, s, H-6"), 4.1 - 5.1 (5H, m, H-2', H-3', H-4', H-5', H-5"), 3.95 (3H, s, -COOCH3). All signals mentioned are present in pairs due to the presence of the D-gulo and D-ido isomers.

^{13}C-NMR (DMSO-d_6) : δ 165.2 (C=O), 152.4/152.7 (C-2), 148.6/149.1 (C-6), 138.0/138.2 (C-4), 135.1/135.4 (C-5), 124.6/124.9 (C-3), 138.5/138.7 and 125.6 -128.5 (aromatic C-atoms), 98.9 - 99.3 (C-6', C-6"), 80.4/81.4 - 66.4 (C-1', C-2', C-3', C-4', C-5'), 52.3 (-OCH3).

DCI-mass spectrometry (NH_3) : m/z = 464 ($[MH]^+$, 100%).

D-gulo/D-ido 3-carbamoyl-5-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (9).

The methyl ester (8) (600 mg, 1.30 mmol) was suspended in CH_3OH (300 ml), cooled to 0°C, and saturated with NH_3. The mixture was stirred at room temperature for 4 days. Evaporation of the solvent gave (9) (580 mg, 100%).

^{1}H-NMR (DMSO-d_6) : δ 9.1 (1H, d, J=1.7 Hz, H-6), 8.8 (1H, d, J=2.2 Hz, H-2), 8.4 (1H, t, H-4), 7.25 - 7.5 (10H, m, aromatic protons), 6.0 (1H, d, J=5 Hz, H-1'), 5.8 (1H, s, H-6'), 5.65 (1H, s, H-6"), 3.9 - 4.9 (5H, m, H-2', H-3', H-4', H-5', H-5"). All signals

mentioned are present in pairs due to the presence of the D-gulo and D-ido isomers.
^{13}C-NMR (DMSO-d_6) : δ 166.4 (C=O), 150.7/151.0 (C-2), 147.4/148.8 (C-6), 136.1/136.3 (C-4), 133.8/134.2 (C-5), 125.1/125.4 (C-3), 138.2/138.5 and 125.3 - 129.3 (aromatic C-atoms), 98.9 - 99.6 (C-6’, C-6”), 80.4/81.6 - 66.7 (C-1’, C-2’, C-3’, C-4’, C-5’).
DCI-mass spectrometry (NH_3) : m/z = 449 ([MH]$^+$, 100%).

D-gulo/D-ido 3-carbamoyl-5-(1-O-mesyl-2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (10).

To a solution of compound (9) (500 mg, 1.12 mmol) in dry pyridine (125 ml), 2 ml CH_3SO_2Cl was added at room temperature. The reaction mixture was stirred for 5 hours and quenched by pouring the mixture in a saturated $NaHCO_3$-solution (300 ml). The aqueous layer was extracted with EtOAc (3x150 ml) and the combined organic layers were dried over $MgSO_4$. After evaporation, a yellow foam was isolated, which was purified by preparative CCTLC (eluant : CH_2Cl_2/CH_3OH (90:10), yielding D-gulo/D-ido 3-carbamoyl-5-(1-O-mesyl-2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine (10) as a white foam (204 mg, 72%).
^{1}H-NMR (acetone-d_6) : δ 9.2 (1H, d, J=1.7 Hz, H-6), 8.9 (1H, d, J=2.2 Hz, H-2), 8.5 (1H, t, H-4), 7.35 - 7.7 (10H, m, aromatic protons), 5.9 (1H, d, J=8.5 Hz, H-1'), 5.8 (1H, s, H-6'), 5.7 (1H, s, H-6"), 3.9 - 4.7 (5H, m, H-2', H-3', H-4', H-5', H-5"), 2.95 (3H, s, $-OSO_2CH_3$). All signals are present in pairs due to the presence of the D-gulo and D-ido isomers.
^{13}C-NMR (acetone-d_6) : δ 167.1 (C=O), 152.3/152.5 (C-2), 149.9/150.5 (C-6), 135.2/135.6 (C-4), 133.6/133.8 (C-5), 125.3/125.6 (C-3), 139.3/139.5 and 125.5 -131.7 (aromatic C-atoms), 79.9/81.6 - 69.2 (C-1', C-2', C-3', C-4', C-5'), 38.8/39.1 ($-OSO_2CH_3$).
DCI-mass spectrometry (NH_3) : m/z = 527 ([MH]$^+$, 100%).

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