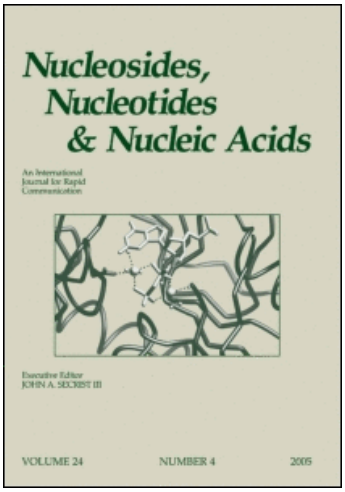

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Synthesis and Biological Evaluation of a Series of Substituted Pyridine-C-Nucleosides. Part V: 3-Chloro-4-(D-Ribofuranosyl)Pyridine and 3-(D-Ribofuranosyl)-2-Pyridone

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SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF SUBSTITUTED PYRIDINE-C-NUCLEOSIDES. PART V: 3-CHLORO-4-(D-RIBOFURANOSYL)PYRIDINE AND 3-(D-RIBOFURANOSYL)-2-PYRIDONE.

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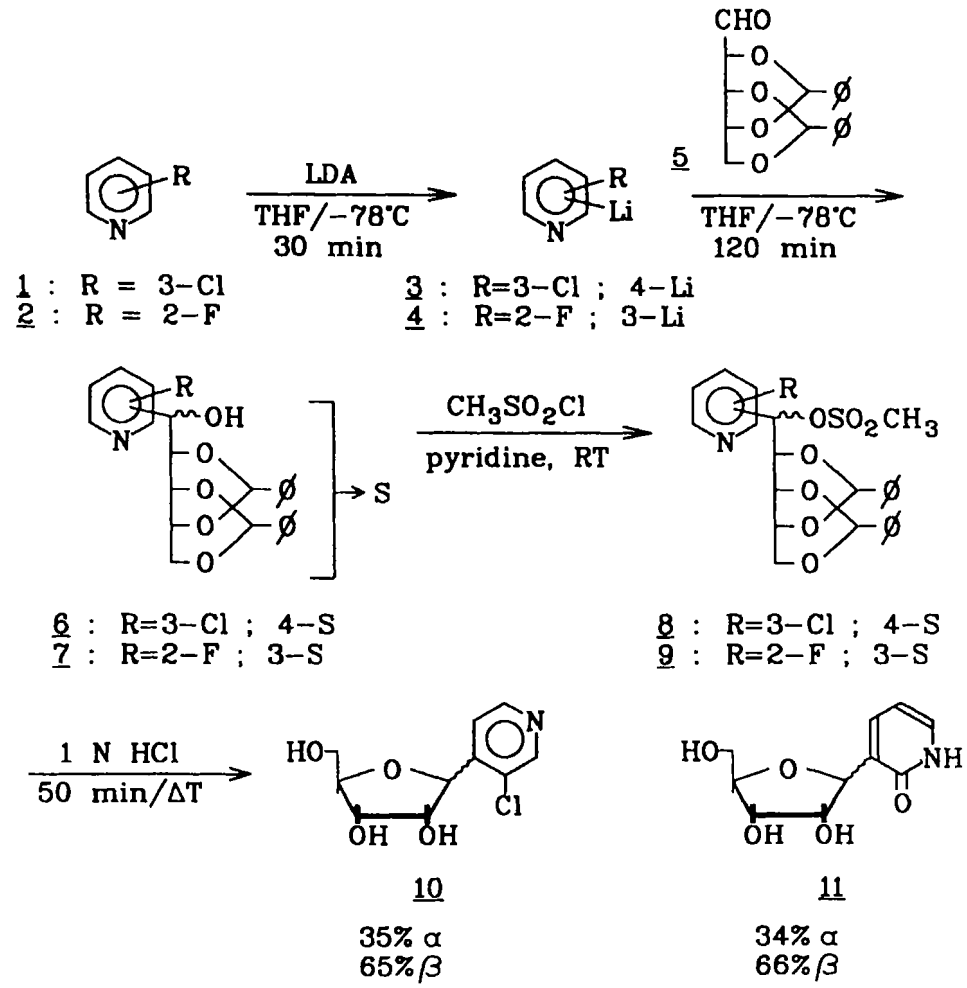
ABSTRACT

The total synthesis of 3-chloro-4-(D-ribofuranosyl)-pyridine and 3-(D-ribofuranosyl)-2-pyridone was elaborated using the appropriate lithiopyridines and 2,4:3,5-di-O-benzylidene-aldehydo-D-ribose. The conformation of these compounds was investigated by 360 MHz ^{1}H-NMR. The compounds were evaluated as α,β-mixtures for their antiviral and cytostatic properties. However, no significant biological activity was found.

I. INTRODUCTION

Since the observation that 3-deazacytidine and 3-deazauridine are able to inhibit RNA-virus replication *in vitro*[1], we have been interested in the synthesis of substituted pyridine-C-nucleosides. More recently, the oncolytic C-nucleoside 2-(β-D-ribofuranosyl)-thiazole-4-carboxamide (tiazofurin) has emerged as a promising new drug with activity against a number of tumors[2]. This activity may be in part due to the resistance of C-nucleosides to enzymatic cleavage of the glycosidic bond, owing to the stability of the carbon-carbon bond. This prompted us to initiate a programme involving the synthesis of pyridine-C-nucleosides structurally related to N-(β-D-ribofuranosyl) nicotinamide. We now wish to report on the synthesis, conformation and biological evaluation of 3-chloro-4-(D-ribofura-nosyl)-pyridine and 3-(D-ribofuranosyl)-2-pyridone[3].



SCHEME 1

II. RESULTS AND DISCUSSION

In previous papers[4,5] we have shown that pyridine-C-nucleosides can be prepared with the aid of pyridyllithiums and 2,4:3,5-di-O-benzylidene-aldehydo-D-ribose.

The crucial step in this total synthesis is the preparation of these lithio intermediates followed by the addition of the sugar synthon.

As shown by several authors[6,7] lithiation of pyridine compounds is highly dependent on the nature of the functional groups on the ring. Hence lithiating reagents have to be chosen with respect to the pyridine derivative used and specific reaction conditions must be rigorously controlled.

As shown by Gribble[8], 2-fluoro- and 3-chloropyridine can be lithiated directly at the C_3- and C_4-positions respectively, provided that lithium diisopropylamide (LDA) in THF (-78°C) is used. By deuterium incorporation, 3-chloropyridine was shown to react regioselectively at the C_4-position (95%) (SCHEME 1)

After a reaction time of 30 minutes, 2,4:3,5-di-O-benzylidene-aldehydo-D-ribose (5) in dry THF was slowly added to the unstable and highly reactive pyridyllithium (3 or 4). The reaction mixture was kept for 2 hours at -78°C and the temperature was allowed to rise to 20°C. The D-allo- and D-altro addition products (6 resp. 7) were isolated in 94% and 92% yield after short column chromatography on RP 1 (eluant : ethylacetate/hexane 30/70). Without any further separation of the D-allo and D-altro isomers, the addition products were immediately mesylated using methane sulphonic acid chloride in dry pyridine for 20 hours. These mesylates (8 or 9) could be isolated by column chromatography using ethylacetate/hexane 80/20 on a RP 1 column (20 cm x 2 cm I.D.). Unidentified impurities remained adsorbed on top of the column. Both products were isolated as white foams with a yield of 80-85%. Subsequent cyclisation of the 4-chloro derivative in 1 N HCl for 50 minutes resulted, as expected, in the formation of 4-(D-ribofuranosyl)-3-chloropyridine (10). However, cyclisation of the D-allo- and D-altro-3-(1-O-mesyl-2,4:3,5-di-O-benzylidene-pentitol-1-yl)-2-fluoropyridine resulted in the formation of 3-(D-ribofuranosyl)-2-pyridone (11). This reaction can be explained by solvolytic displacement of the fluorine atom during the acid catalysed cyclisation. After 50 minutes, the reaction mixtures were extracted with chloroform. Neutralisation with conc. NH_4OH gave the crude nucleosides which were purified by affinity chromatography on a Affigel 601 boronate (Biorad) column. The pyridine-C-nucleosides 10 and 11 were isolated after lyophilisation as sirups. Yields : 88% 10 and 86% 11. Their purity was checked by DLI/LC-MS[9].

III. 360 MHz ^{1}H-N.M.R.

The 360 MHz ^{1}H-NMR-spectra of 3-chloro-4-(D-ribofuranosyl)pyridine (10) and 3-(D-ribofuranosyl)-2-pyridone (11) were recorded in CD_3OD-solutions with TMS as internal reference and are summarized in TABLES 1 and 2.

Assignment of the anomeric configuration was done with the aid of the syn-upfield rule ($\delta H_{1'\alpha} > \delta H_{1'\beta}$), which is in general applicable to ribofuranosyl derivatives. For both nucleosides the $H_{1'}$-proton of the α-isomer resonated at lower field than the proton of the corresponding β-isomer. However, since there is no general rule for the α/β-assignment based on the chemical shift differences between the isomers for protons other than $H_{1'}$, the assignment of $H_{2'\alpha}$ versus $H_{2'\beta}$, $H_{3'\alpha}$ versus $H_{3'\beta}$, etc., was done by the observed difference in intensity in the spectrum.

TABLE 1 :360 MHz ^{1}H-NMR-data of compounds 10 and 11, δ-values relative to TMS.

		H_2	H_3	H_4	H_5	H_6
10	α	8.50(d)	-	-	7.60(d)	8.27(d)
10	β	8.50(d)	-	-	7.82(d)	8.30(d)
11	α	-	-	7.71(d)	6.40(d)	7.36(d)
11	β	-	-	7.74(d)	6.40(d)	7.38(d)

TABLE 2 :360 MHz-^{1}H-NMR-data of compounds 10 and 11: sugar region. δ-Values relative to TMS and coupling constants.

		H_1'	H_2'	H_3'	H_4'	H_5'	H_5"
10	α	5.36(d)	4.52(t)	4.31(dd)	4.07(m)	3.89(dd)	3.69(dd)
10	β	5.19(d)	4.0(m)	4.00(m)	4.00(m)	3.93(dd)	3.78(dd)
11	α	5.09(d)	4.38(t)	4.28(dd)	4.0(m)	3.86(dd)	3.67(dd)
11	β	4.82(d)	4.0(m)	4.13(t)	4.0(m)	3.81(dd)	3.66(dd)
		J(1',2')	J(2',3')	J(3',4')	J(4',5')	J(4',5")	J(5',5")
10	α	3.4	4.4	8.6	2.5	4.6	-12.1
10	β	2.8	[a]	[a]	2.5	4.1	-11.1
11	α	3.0	4.3	8.4	2.6	4.6	-11.6
11	β	4.6	[a]	[a]	2.7	3.3	-12.1

[a] could not be determined.

The conformation of the D-ribofuranosyl moiety, expressed as a %N/%S equilibrium, was calculated according to the data of Altona[10,11]. For product 10 we found a 28%S/72%N-type equilibrium. Product 11 showed a nearly 50/50 ratio of both conformers. The conformation around the C_4'-C_5'-linkage expressed as a %gg, %gt and %tg equilibrium was calculated according to the data of Davies[12]. Both nucleosides 10 and 11 predominantly exist in a gg-conformation, which is a general rule for nucleosides. (Table 3). For all the isomers, the following tendency was observed :

$$\% \text{ gg} >> \% \text{ gt} > \% \text{ tg}$$

Comparison of the % gg in the same C-nucleoside (α and β) showed that:

$$\% \text{ gg}_\beta > \% \text{ gg}_\alpha$$

IV. 25 MHz ^{13}C-N.M.R.

The 25 MHz ^{13}C-NMR-spectra of 10 and 11 were obtained from D_2O-solutions. The results are gathered in TABLE 4 with DMSO as internal standard. (FIG. 2).

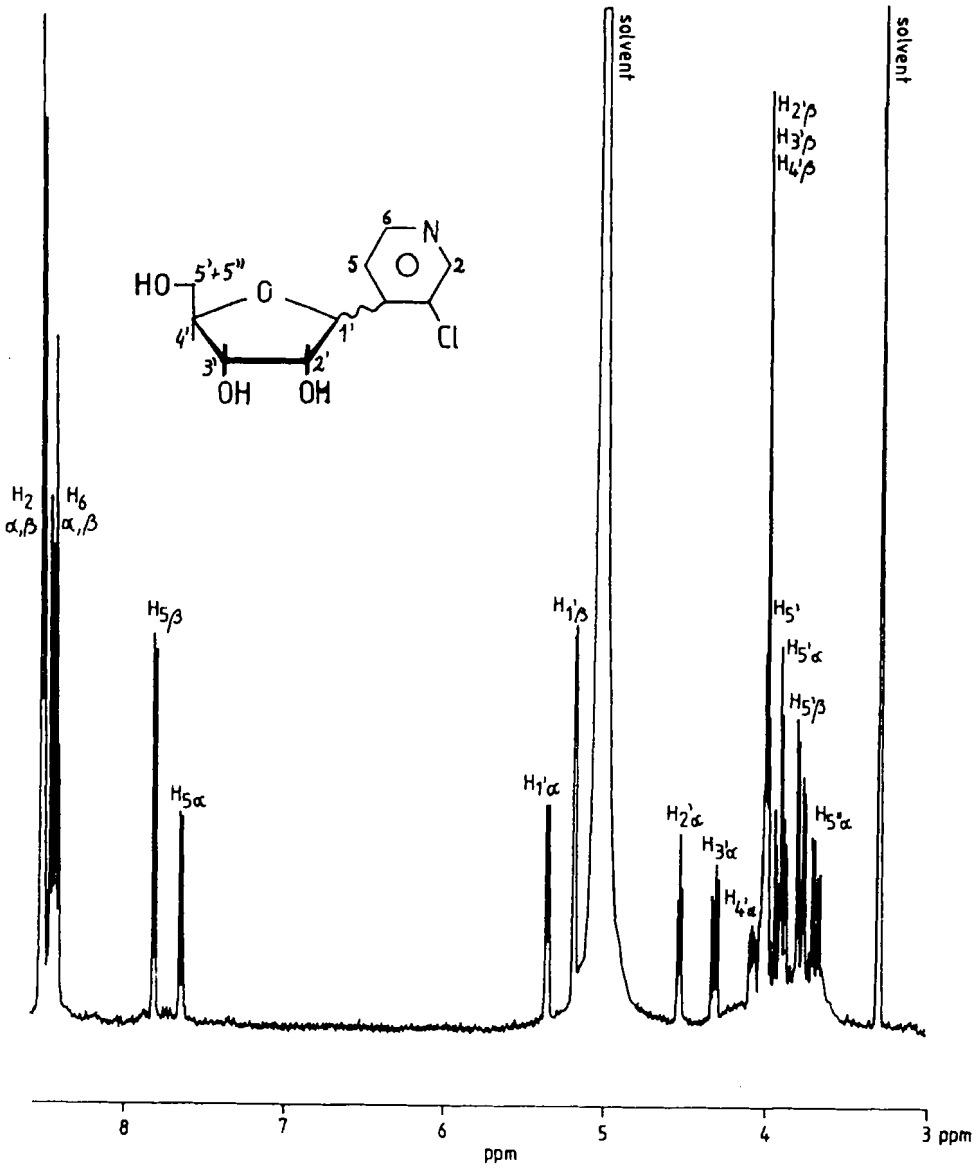


FIG. 1 : 360 MHz ^{1}H-NMR of 3-chloro-4-(D-ribofuranosyl)pyridine (10).

TABLE 3.

		%gg	%gt	%tg
10	α	69	26	5
10	β	74	21	5
11	α	67	26	7
11	β	79	14	7

TABLE 4 : ^{13}C-NMR data

	C_2	C_3	C_4	C_5	C_6	C_1'	C_2'	C_3'	C_4'	C_5'
10 α	159.0	113.7	135.0	125.7	143.5	81.4	71.8	72.1	77.7	61.6
10 β	159.8	113.5	135.5	126.7	143.1	83.8	74.7	71.1	79.7	61.6
11 α	139.5	131.3	157.3	125.1	138.5	80.8	70.8	70.6	79.0	60.1
11 β	139.9	131.8	157.5	124.0	139.1	82.0	75.3	69.3	80.7	60.4

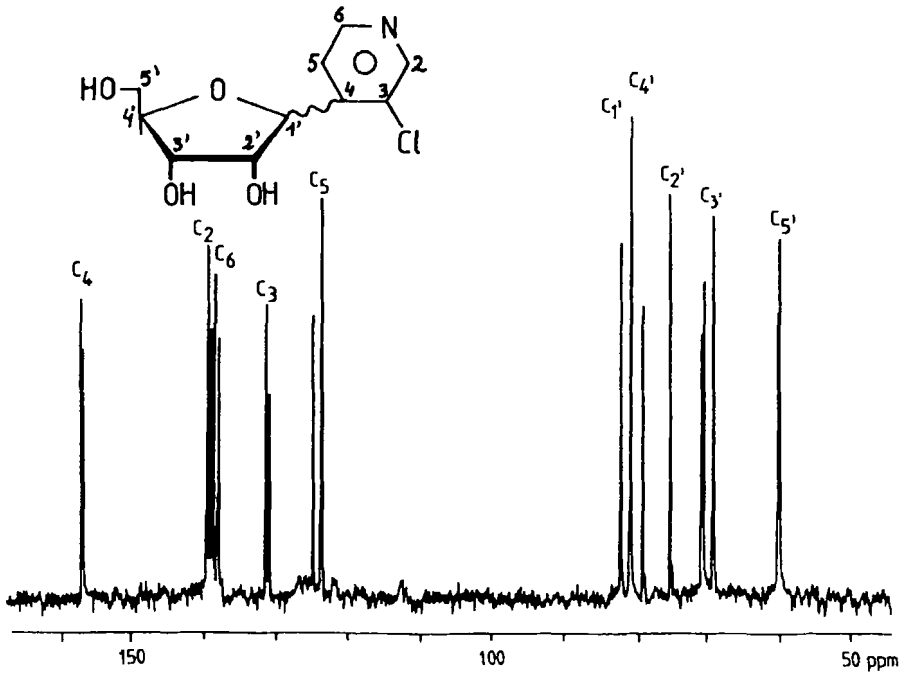


FIG. 2 : ^{13}C-NMR spectrum of 3-chloro-4-(D-ribofuranosyl)pyridine (10)

The assignment of the carbon atoms to the α- or the β-anomer was accomplished by taking into account the relative difference in intensity between the two isomers. For the assignment in the aromatic region chemical shift increments were used. For the sugar moiety, the signal located around 60 ppm was assigned to the primary C_5, carbon-atom. Location of C_2, and C_3, was done by using the data recently published by Beier[13]. The assignment of C_1, and C_4, was accomplished in analogy with previously published data.

V. BIOLOGICAL STUDIES : *IN VITRO* ANTIVIRAL AND CYTOSTATIC EFFECTS.

Nucleosides 10 and 11 were evaluated for their inhibitory effect on the replication of several viruses [i.e. herpes simplex virus type 1 (KOS), herpes simplex virus type 2 (G), vaccinia virus and vesicular stomatitis virus (VSV) in primary rabbit kidney cells ; VSV, Coxsackie virus type B4 and polio virus type 1 in HeLa cells ; parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie virus type B4 and Semliki forest virus in Vero cells ; human immunodeficiency virus (HIV) in MT4 cells] and the proliferation of murine leukemia L1210, murine mammary carcinoma FM3A, human B-lymphoblast Raji and human T-lymphoblast Molt/4F cells *in vitro*. No activity was found against any of the viruses tested, even ith compound concentrations up to 100, 200 or 400 μg/ml. Nucleoside 11 was not cytostatic at 1000 μg/ml against the different tumor cell lines, while nucleoside 10 effected a 50% inhibition of the proliferation of L1210, FM3A, Raji and Molt/4F cells at a concentration of 307, > 1000, 325, and 363 μg/ml, respectively.

VI. EXPERIMENTAL

a. Products: 2-Fluoro- and 3-chloropyridine were purchased from Janssen Chimica (Belgium).
2,4:3,5-di-O-benzylidene-aldehydo-D-ribose was prepared according to Zinner[14].

b. Methods: 360 MHz-^{1}H-NMR spectra were recorded on a BRUKER WH-360-NMR spectrometer (R.U.Ghent). ^{13}C-NMR spectra were recorded on a Jeol JNM PFT-PS-100 spectrometer.

c. Synthesis of D-allo/D-altro 4-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)-3-chloro-pyridine (6) and D-allo/D-altro 3-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)-2-fluoropyridine (7).

A three necked flask of 100 ml, equipped with a dropping funnel, $CaCl_2$ tube and dry N_2-inlet system, was filled with 3,7 ml dry diisopropylamine (26,4 mmol) (distilled from CaH_2) and 20 ml freshly distilled (from $LiAlH_4$) THF. The solution was cooled in a CO_2/acetone bath to -78°C, and 16,5 ml of BuLi (1,6 M in hexane, 30 mmol) was added under stirring. Stirring was continued for another 20 minutes at -78°C. Then, a solution of 3-chloropyridine (26,4 mmol in 5 ml dry THF) or 2-fluoropyridine (26,4 mmol in 5 ml dry THF) was added over a period of 15 minutes.

After addition of the pyridine compound, the mixture was stirred for 30 minutes at -78°C. Then a solution of 2,4:3,5-di-O-benzylidene-aldehydo-D-ribose in 20 ml THF was added over a period of 30 minutes (-78°C) (17.6 mmol). The temperature was then allowed to rise to room temperature

overnight. After the addition of H_2O (50 ml) and CH_3OH (20 ml) the mixture was extracted with $CHCl_3$ (3 x 50 ml). The $CHCl_3$-layer was dried on $MgSO_4$, filtered off and evaporated. The resulting sirup was chromatographed on a short RP1 column using ethylacetate (hexane (3/7). During this purification procedure a dark brown residue remained on the column. The eluant was evaporated and the D-allo/D-altro adducts were isolated as yellow sirups (6: 94% 7: 92% yield).

d. Mesylation of D-allo/D-altro 4-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)-3-chloropyridine (6) and D-allo/D-altro 3-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)-2-fluoropyridine (7).

To a solution of 6 (7) (2 mmol) in dry pyridine (20 ml), CH_3SO_2Cl (6 mmol) was slowly added at room temperature. Stirring was continued for 24 hrs under exclusion of moisture. After the reaction mixture was poured into an excess of saturated $NaHCO_3$, the mesylates were extracted with $CHCl_3$ (3 x 50 ml). The $CHCl_3$-layer was dried over $MgSO_4$ and, after filtration, evaporated. The resulting sirup was purified on a RP 1 column (MERCK, Kieselgel 60-silaniert, 70-230 Mesh) with ethyl acetate/hexane (4/1). Brown unidentified compounds remained on the column. After evaporation of the solvent, the D-allo/D-altro mesylates 8(9) were obtained as white foams.
Yields : 80-85%.

e. Synthesis of 3-chloro-4-(D-ribofuranosyl)pyridine(10) and 3-(D-ribofuranosyl)-2-pyridone(11).

In a flask of 100ml, equipped with a reflux condenser 1 N HCl (20 ml) was added to 500 mg of D-allo/D-altro mesylate 8 (9). After the mesylate was completely dissolved by warming up, the solution was refluxed for 50 min. Then the reaction mixture was cooled in an ice bath and extracted with $CHCl_3$. The aqueous layer was then evaporated, the residue redissolved in H_2O and neutralised with aq. NH_3. This solution, containing the C-nucleosides, was cleaned up by affinity chromatography on an Affigel 601-boronate column according to the procedure previously described by us[5]. The pyridine-C-nucleosides 10 and 11 were obtained as sirups. Yields : 10 : 88% ; 11:96%.

f. Biological evaluation.

Antitumor and antiviral effects were determined as described previously[5,15].

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