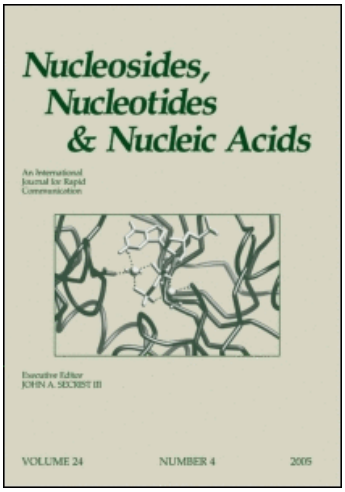

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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-(2-DEOXY-D-ERYTHRO-PENT-1-ENOFURANOSYL)PYRIDINE C-NUCLEOSIDES.

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ABSTRACT.

2-(2-Deoxy-D-erythro-pent-1-enofuranosyl)pyridine and its methyl analogues have been prepared by treatment of the corresponding 2',3'-O-isopropylidene-D-ribofuranosyl derivatives with several bases such as sodium amide, *tert.*BuOK, EtONa, lithium tetramethylpiperidide (LTMP) and phenyl lithium (PhLi). PhLi and *tert.*BuOK gave the best results. The products thus obtained showed cytostatic activity against human tumor cell lines, in particular MT-4, a human T-lymphocyte cell line. No antiviral activity was noted at subtoxic concentrations.

I. INTRODUCTION.

Within the series of purine and pyrimidine nucleosides, 2',3'-dideoxy-nucleosides and 2',3'-dideoxy-2',3'-didehydronucleosides have proven to be potent inhibitors of HIV-1[1-5] (Human Immunodeficiency Virus Type 1).

The synthesis of these compounds is well documented and various strategies have been developed in order to introduce a double bond between C-2' and C-3'.

To our knowledge only a few nucleosides containing a 2-deoxy-D-erythro-pent-1-enofuranosyl moiety have been reported in the literature[6-8] (FIGURE 1).

Some of these structures were the result of an undesired side reaction involving the elimination of a protecting group on the sugar moiety[9,10]. Little, if any, information is available on their biological activity : only structure 3 was reported to inhibit MuLV cell replication[11].

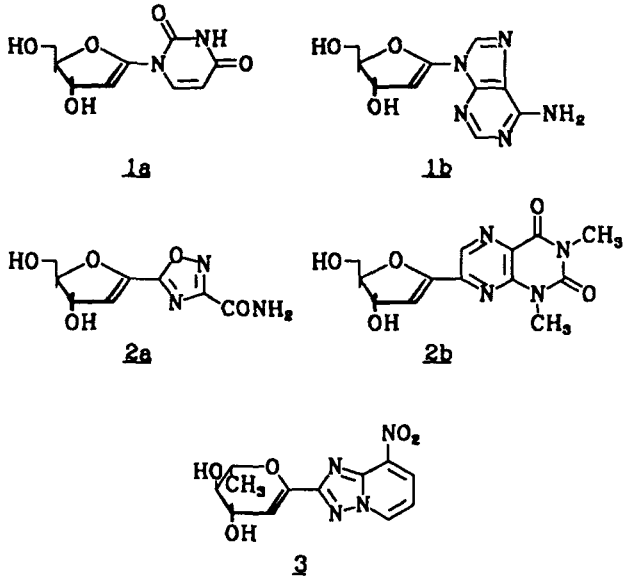


FIGURE 1. : Examples of N-glycosylated (1a,1b) or C-glycosylated (2a,2b) 2-deoxy-D-erythro-pent-1-enofuranosyl nucleosides and of a C-glycosylated (3) 2,6-dideoxy-L-arabino-hex-1-enopyranosyl nucleoside.

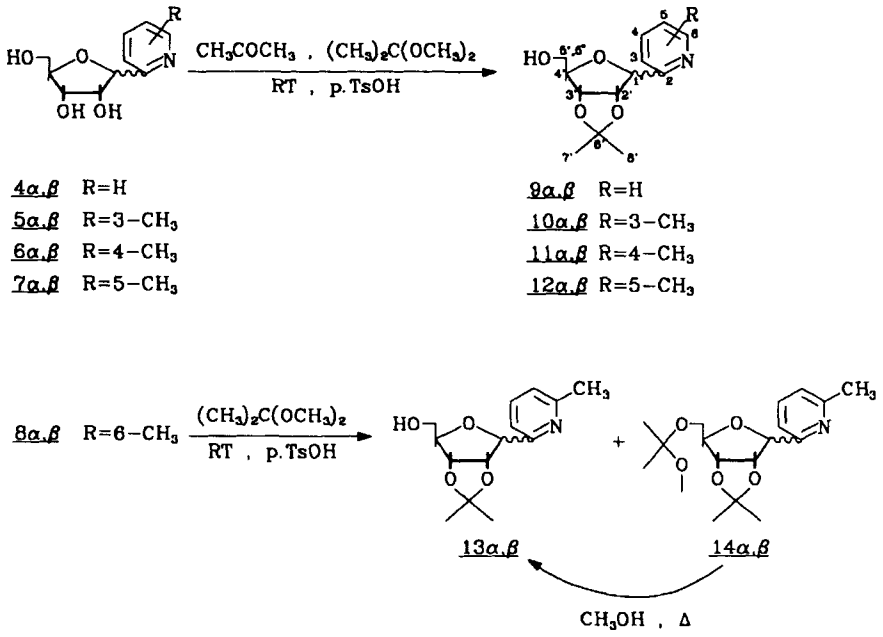
Our laboratory has been involved in the synthesis of pyridine C-nucleosides in attempts to develop novel compounds with cytostatic and/or antiviral properties. Several of such compounds have been reported by us[12-16] and others[17-19], some of them show modest *in vitro* cytostatic activity.

We now report on the synthesis and biological activity of a novel series of pyridine C-nucleosides characterized by the presence of a double bond in the sugar moiety.

II. RESULTS AND DISCUSSION.

II.a. 2',3'-O-Isopropylidene-D-ribofuranosyl pyridine C-nucleosides.

It has been reported that ethyl 3-(2,3-O-isopropylidene-5-O-trityl-β-D-ribofuranosyl)-3-oxopropanoate upon treatment with sodium ethoxide yields ethyl 3-(2-deoxy-5-O-trityl-D-erythro-pent-1-enofuranosyl)-3-oxopropanoate[20]. We have now investigated the behavior of a series of 2',3'-O-isopropylidene-pyridine C-nucleosides upon treatment with a variety of strong bases (phenyl lithium (PhLi), lithium tetramethylpiperidide (LTMP), *tert.*BuOK, EtONa and $NaNH_2$), as a possible route to the introduction of a double bond between C-1' and C-2'. The starting materials (**4** - **8**) were prepared according to a procedure previously described by us[13] but the anomeric mixture thus obtained was separated with the aid of preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron® (mobile phase: CH_2Cl_2/CH_3OH (9/1 v/v)). This method was applied for the methyl substituted compounds **5**-**8**. In the case of 2-D-ribofuranosylpyridine (**4**) the α,β-separation was achieved by using semi-preparative HPLC (LiChrosorb 10RP8 25cm x 9.4 mm I.D.; mobile phase: 0.1 N $HCOONH_4/CH_3OH$ (97/3 v/v)). Both α- and β-D-ribofuranosyl-pyridine C-nucleosides were then separately converted to the corresponding 2',3'-isopropylidene derivatives (**9**-**12**) with acetone/2,2-dimethoxypropane (20/1 v/v), using p-toluene sulphonic acid (p-TsOH) as a catalyst (SCHEME 1). The yields varied from 82% to 92%. This method was chosen because the use of 100% 2,2-dimethoxypropane/p-TsOH as a reagent on α and β 6-methyl-D-ribofuranosylpyridine led to the formation of 2-[2,3-0-isopropylidene-5-0-(2-methoxyisopropyl)-D-ribofuranosyl]-6-methylpyridines **14α** and **14β** (yield: **14α** :45% ; **14β** :12%) in addition to the desired 2',3'-0-isopropylidene derivatives **13α** (42%) and **13β** (79%). However, if necessary, **14α** and **14β** can be converted to **13α** and **13β** by refluxing the former compounds in methanol for 5 days.

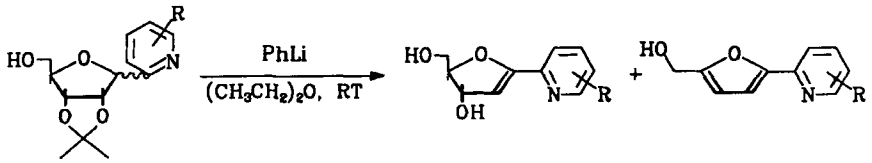


SCHEME 1.

II.b. 1',2'-Unsaturated pyridine C-nucleosides.

In order to optimize the yields of the 1',2'-unsaturated pyridine C-nucleosides (**15** to **19**), the elimination reaction depicted in SCHEME 2 was studied under different experimental conditions. If **13α** and **13β** were separately treated with 3 eq. LTMP in 1,2-dimethoxyethane (DME) at -78°C for two hours, 18% and 16% respectively of compound **19**, was obtained. The furan structure was isolated in 1% and 2%, and starting material was recovered in 70% and 74% yield. Reaction of **9β** with 4.5 eq. LTMP in DME yielded 36% of **15**, 6% furan derivative **20** and 55% starting material. Although these reactions were performed on different compounds (**13α**, **13β** and **9β**) these results could indicate that a 50% increase in lithio reagent gives better yields.

However the use of substantially more LTMP (6 eq.) again decreased the yield (21%). Probably, since LTMP is a sterically hindered base, the deprotonation of



9α,β	15 R=H	20 R=H
10α,β	16 R=3-CH_3	21 R=3-CH_3
11α,β	17 R=4-CH_3	22 R=4-CH_3
12α,β	18 R=5-CH_3	23 R=5-CH_3
13α,β	19 R=6-CH_3	24 R=6-CH_3

SCHEME 2.

C-1' is slow and at high concentrations undesirable side reactions begin to prevail. This is a plausible explanation since alkyl substituted lithio amides are known to add to pyridine derivatives inducing ring opening reactions even at -70°C[21].

Treatment of **10β** with 3 eq. *tert.*BuOK in *tert.*BuOH (1N to 0.025N solutions) at 105°C and the use of different reaction times (80 min. for a 1N *tert.*BuOK solution; 6 hrs. for a 0.025N *tert.*BuOK solution) resulted in the formation of **16** in yields varying from 72% to 74%.

$NaNH_2$ in DME yielded only 20% of **16** while reaction with EtONa yielded 70% but only after 7 days of reflux.

However, following the reaction of the 2',3'-O-isopropylidene compounds with 3.3 eq PhLi in dry diethyl ether at room temperature for 15 min. 82% to 95% yields of the corresponding 1',2'-unsaturated C-nucleosides **15** to **19** were obtained. In each case the formation of the corresponding 2-(5-hydroxymethyl-fur-2-yl)pyridines **20** - **24** could not be avoided despite careful (pH ≥ 7.5) work-up conditions and immediate isolation by CCTLC (mobile phase: CH_2Cl_2/ CH_3OH 97/3). However these furan derivatives were only present in minor amounts (1% - 2%).

From the results described above one can conclude that for the series of compounds described in this paper both PhLi and *tert.*BuOK are the reagents of

choice. Furthermore, it is noteworthy that the elimination of acetone from the 2',3'-O-isopropylidene C-nucleosides is independent of the anomeric configuration of the starting material. Therefore time consuming α/β separations, prior to the synthesis of the acetonides, can be omitted.

II.c. Stability of 1',2'-unsaturated pyridine C-nucleosides.

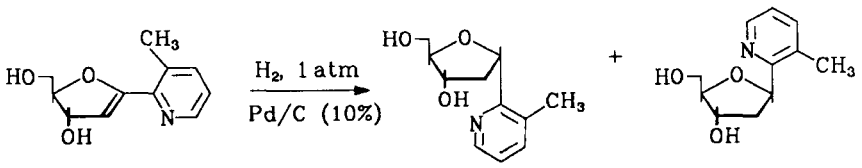
The observation that an acidic work-up of compound **15** led to a large amount of furan derivative **20** and the fact that the 1',2'-unsaturated pyridine C-nucleosides - in general - have a tendency to form the corresponding furan derivatives by eliminating H_2O even at pH = 7.5 (see par II.b.), prompted us to study their stability in solution, prior to the biological evaluations.

Therefore the compounds **15** to **19** were dissolved in a 0.01M phosphate buffer (pH = 7.5; T = 37°C) and stirred for several days. Aliquots were taken at different time intervals and analyzed by HPLC (LiChrosorb 10RP8, 25 cm x 4.6 mm I.D., flow-rate: 2 ml/min, detection UV: 296 nm).

The results are summarized in TABLE 1 :

TABLE 1. : Stability measurements of compounds **15** to **19**.

Compound	eluant : CH_3OH/H_2O	t_R (1',2'-ene) minutes	t_R (furan) minutes	half-life time (days)
15	2/3	2.5	5.5	6.7
16	2/3	4.5	7.7	4.9
17	2/3	4.5	7.5	5.5
18	2/3	4.5	7.3	3.3
19	1/1	2.2	3.7	3.1



16 25α 25β

Yield : 25% Yield : 61%

SCHEME 3.

From these data one can see that the stability of the compounds decreases in the order H > 4-Me ≥ 3-Me > 5-Me ≥ 6-Me and that under the above mentioned conditions the compounds remain stable long enough for biological evaluation.

II.d. Biological Evaluation.

Compounds **15** to **19**, **24**, **25α** and **25β** were evaluated for their cytostatic activity against murine leukemia L1210 cells and human B-lymphoblast Raji, T-lymphoblast Molt/4F and T-lymphocyte MT-4 cells. Compounds **25α** and **26β** were obtained by catalytic hydrogenation (Pd/C in EtOH at 1 atm) of compound **16** (SCHEME 3).

As a rule, the test compounds proved less cytostatic against murine L1210 cells than human B- or T-cells. Among the human cell lines, MT-4 cells proved the most sensitive to the inhibitory effect of the 1',2'-unsaturated pyridine C-nucleosides on cell proliferation. The 50% inhibitory concentrations (IC_{50}) for compounds **15** to **19** ranged from 7 to 38 μg/ml. In particular, compound **19** (containing a 6-methyl substituent in the pyridine ring) was most effective (IC_{50} : 7 μg/ml). The 1',2'-saturated (2'-deoxyribosyl) pyridine C-nucleosides were devoid of cytostatic activity against any of the tumor cell lines tested.

All compounds mentioned in TABLE 2 were also evaluated for their inhibitory effects on the replication of a number of DNA viruses [herpes simplex virus type 1 (strain KOS) and type 2 (strain G), and vaccinia virus] and RNA viruses

TABLE 2. : Inhibitory effects of test compounds on the growth of murine leukemia L1210, human B-lymphoblast Raji, human T-lymphoblast Molt/4F and human T-lymphocyte MT-4 cells. 50% Inhibitory concentration* (μg/ml).

Compound	L1210	Raji	Molt/4F	MT-4
15	107 ± 5	24 ± 1.2	27 ± 0.6	15 ± 0.3
16	93 ± 0.3	-	77 ± 2.1	38 ± 23
17	64 ± 1.0	17 ± 9.7	19 ± 1.6	12 ± 0.8
18	90 ± 3.9	-	42 ± 22	16 ± 1.0
19	394 ± 108	51 ± 2.5	43 ± 1.0	7.0 ± 0.33
24	137 ± 21	-	122 ± 0.7	> 100
25α	> 200	-	> 200	> 200
25β	> 200	-	> 200	> 200

* : Concentration required to inhibit tumor cell proliferation by 50%.

(vesicular stomatitis virus, Coxsackie virus B4, polio virus type 1, parainfluenza virus type 3, reovirus type 1, Sindbis virus, Semliki forest virus and human immunodeficiency virus type 1. No antiviral activity was noted at subtoxic concentrations of the test compounds (data not shown).

II.e ^{1}H and ^{13}C NMR data (Tables 3-13).

For D-ribofuranosyl C-nucleosides protected with the 2',3'-O-isopropylidene group, there are two basic criterions which have been successfully utilized for assignment of anomeric configuration: the Imbach[22] and the chemical shift (H-1') rule[22].

TABLE 3. : 360 MHz ^{1}H NMR data ($CDCl_3$) of the α-2',3'-O-isopropylidene derivatives (**9α** - **13α**).

	9α	10α	11α	12α	13α
H-1'	5.25 d	5.37 d	5.29 d	5.24 d	5.23 d
$J_{H-1',H-2'}$	4.2	4.9	4.2	4.1	4.0
H-2'	5.06 dd	5.12 dd	5.06 dd	5.03 dd	5.04 dd
$J_{H-2',H-3'}$	5.9	6.2	6.0	6.0	5.9
H-3'	4.81 dd	4.81 dd	4.84 dd	4.81 dd	4.81 dd
$J_{H-3',H-4'}$	0.9	1.6	0.9	1.1	1.1
H-4'	4.38 t	4.57 m	4.36 t	4.36 t	4.38 t
$J_{H-4',H-5'}$	5.4	4.1	4.9	5.3	5.5
H-5'	3.76 d	3.81 dd	3.78 d	3.76 d	3.76 d
H-5''	3.76 d	3.76 dd	3.78 d	3.76 d	3.76 d
$J_{H-5',H-5''}$	---	-11.7	---	---	---
$J_{H-4',H-5''}$	5.4	6.2	4.9	5.3	5.5
H-3	7.56 d	---	7.39 s	7.45 s	7.06 d
$J_{H-3,H-4}$	7.9	---	---	8.0	7.6
H-4	7.70 m	7.44 dd	---	7.52 dd	7.60 t
$J_{H-4,H-5}$	7.5	7.6	---	---	7.7
H-5	7.19 dd	7.11 dd	7.01 d	---	7.37 d
$J_{H-5,H-6}$	4.9	4.8	5.1	---	---
H-6	8.52 m	8.46 dd	8.35 d	8.34 d	---
$J_{H-4,H-6}$	1.6	1.2	---	2.0	---
H-7	---	2.40 s	2.36 s	2.31 s	2.54 s
H-7'	1.37 s	1.28 s	1.37 s	1.38 s	1.41 s
H-8'	1.27 s	1.26 s	1.26 s	1.26 s	1.28 s
$\Delta\delta_{H-7',H-8'}$	0.10	0.02	0.11	0.12	0.13

TABLE 4. : 360 MHz ^{1}H NMR data ($CDCl_3$) of the β-2',3'-O-isopropylidene derivatives (**9β** - **13β**).

	9β	10β	11β	12β	13β
H-1'	5.04 d	5.42 d	4.99 d	5.01 d	5.03 m
$J_{H-1',H-2'}$	3.9	3.8	4.0	3.8	3.9
H-2'	4.82 dd	4.78 dd	4.81 dd	4.80 t	4.83 dd
$J_{H-2',H-3'}$	5.9	5.9	5.8	5.9	5.7
H-3'	4.99 dd	4.98 dd	4.99 d	4.99 d	5.03 m
$J_{H-3',H-4'}$	1.5	1.7	---	---	1.1
H-4'	4.51 m	4.53 m	4.52 s	4.50 s	4.54 d
$J_{H-4',H-5'}$	2.0	2.1	1.9	---	1.8
H-5'	3.97 dd	4.01 dd	3.98 dd	3.96 d	4.01 dd
H-5''	3.70 dd	3.69 dd	3.69 dd	3.68 d	3.71 d
$J_{H-5',H-5''}$	-12.5	-12.4	-12.5	-12.5	-12.5
$J_{H-4',H-5''}$	2.1	2.0	2.0	---	---
H-3	7.24-7.31 m	---	7.11 s	7.17 d	7.08 d
$J_{H-3,H-4}$	7.7	---	---	7.8	7.6
H-4	7.71 dt	7.51 dd	---	7.50 d	7.59 t
$J_{H-4,H-5}$	7.7	7.7	---	---	7.6
H-5	7.24-7.31 m	7.16 dd	7.10 d	---	7.12 d
$J_{H-5,H-6}$	4.8	4.8	5.0	---	---
H-6	8.57 bd	8.41 dd	8.41 d	8.39 s	---
$J_{H-4,H-6}$	1.7	1.1	---	---	---
H-7	---	2.40 s	2.36 s	2.33 s	2.55 s
H-7'	1.62 s	1.64 s	1.63 s	1.62 s	1.63 s
H-8'	1.35 s	1.36 s	1.36 s	1.35 s	1.36 s
$\Delta\delta_{H-7',H-8'}$	0.27	0.28	0.27	0.27	0.27

TABLE 5. : 500 MHz ^{1}H NMR data ($CDCl_3$) of the 2-(2-deoxy-D-erythro-pento-1-enofuranosyl)-pyridine C-nucleosides (**15** - **19**).

	15	16	17	18	19
H-2'	5.91 d	5.53 d	5.88 d	5.83 d	5.89 d
H-3'	4.87 t	4.87 t	4.85 t	4.85 t	4.85 t
$J_{H-2',H-3'}$	2.9	2.8	2.9	2.8	2.7
H-4'	4.50 m	4.45 m	4.49 m	4.48 m	4.48 m
$J_{H-3',H-4'}$	3.3	3.3	3.3	3.3	3.0
H-5'	3.68 m	3.68 m	3.67 m	3.67 m	3.67 m
$J_{H-4',H-5'}$	6.2	6.3	6.3	6.5	6.2
H-5''	3.64 m	3.64 m	3.66 m	3.64 m	3.63 m
$J_{H-4',H-5''}$	5.3	5.3	5.3	5.1	5.1
$J_{H-5',H-5''}$	-11.9	-12.1	---	-11.9	-11.8
H-3	7.74 d	---	7.60 s	7.63 d	7.23 d
$J_{H-3,H-4}$	7.9	---	---	8.0	7.7
H-4	7.86 dt	7.71 dd	---	7.67 dd	7.71 t
$J_{H-4,H-5}$	7.6	7.8	---	---	7.8
H-5	7.37 m	7.31 dd	7.23 d	---	7.54 d
$J_{H-5,H-6}$	4.9	4.8	5.1	---	---
H-6	8.53 m	8.38 dd	8.37 d	8.37 d	---
$J_{H-4,H-6}$	1.8	1.5	---	2.1	---
$J_{H-3,H-5}$	1.2	---	---	---	---
H-7	---	2.49 s	2.40 s	2.36 s	2.55 s

TABLE 6. : 360 MHz ^{1}H NMR data ($CDCl_3$) of the furan compounds (**20** - **24**).

	20	21	22	23	24
H-2'	6.99 d	6.86 d	6.98 m	6.93 d	6.97 d
H-3'	6.43 d	6.46 d	6.42 d	6.41 d	6.40 d
$J_{H-2',H-3'}$	3.3	3.3	3.3	3.3	3.3
H-5',5''	4.67 s	4.72 s	4.70 s	4.68 s	4.69 s
H-3	7.68-7.71 m	---	7.53 s	7.59 d	7.02 d
H-4	7.68-7.71 m	7.55 d	---	7.52 dd	7.59 t
$J_{H-3,H-4}$	8.0	---	---	7.8	7.6
H-5	7.16 m	7.12 dd	6.99 m	---	7.49 d
$J_{H-4,H-5}$	5.9	7.7	---	---	7.8
H-6	8.59 d	8.52 dd	8.45 d	8.42 d	---
$J_{H-5,H-6}$	4.8	4.7	5.0	---	---
$J_{H-4,H-6}$	1.7	1.5	---	2.1	---
$J_{H-3,H-5}$	1.7	---	---	---	---
H-7	---	2.55 s	2.39 s	2.35 s	2.59 s

All new C-nucleoside acetonides (**9** - **14**) obeyed Imbach's rule ($\Delta\delta CH_3\alpha < 0.15$ ppm and $\Delta\delta CH_3\beta > 0.15$ ppm) (TABLES 3 and 4). This is not as obvious as it seems because at least one anomeric pair of pyridine C-nucleosides is known to be in violation with this methyl resonance criterion. Indeed, Fourey et al.[23] reported a $\Delta\delta CH_3$ value of 0.18 ppm for 2-(methylthio)-5-(2',3'-O-isopropylidene-α-D-ribofuranosyl)pyridine, whereas the β-isomer showed a $\Delta\delta CH_3$ of 0.26 ppm. Pompon et al. offered an explanation for this exception in terms of conformation[24].

The chemical shifts for the anomeric protons (H-1') have been shown to be useful both *in N-nucleoside* and in C-nucleoside anomeric assignments. This method relies upon the observation that the α-anomeric proton consistently appears at

TABLE 7. : 500 MHz ^{1}H NMR data of the 2-(2-deoxy-D-ribofuranosyl)-3-methyl-pyridine C-nucleosides (**25α,β**).

	25α (CD_3OD)	25β (CD_3OD)	25α (DMSO)	25β (DMSO)
H-1'	5.53 dd	5.49 dd	5.30 t	5.34 dd
H-2'β	2.62 dq	2.17 m	2.42 m	2.40 m
H-2'α	2.17 dt	2.25 m	2.25 m	2.02 m
$J_{H\text{-}2'\beta,H\text{-}2'\alpha}$	-13.3	-13.1	-12.7	- 12.8
H-3'	4.34 m	4.44 m	4.17 m	4.26 m
H-4'	4.06 m	3.99 m	3.79 m	3.83 m
H-5'	3.63 m	3.83 m	3.472 m	3.47 m
H-5''	3.59 m	3.66 dd	3.415 m	3.39 m
$J_{H\text{-}4',H\text{-}5'}$	4.5	4.8	3.3	4.6
$J_{H\text{-}4',H\text{-}5''}$	5.0	5.0	3.5	4.4
$J_{H\text{-}5',H\text{-}5''}$	-11.8	-11.8	-12.2	-11.5
OH-3'	---	---	5.7 bs	5.05 d
OH-5'	---	---	4.64 t	5.20 t
$J_{H\text{-}3',OH\text{-}5'}$	---	---	---	4.1
$J_{H\text{-}5',OH\text{-}5'}$	---	---	5.6	4.4
$J_{H\text{-}5'',OH\text{-}5'}$	---	---	5.8	7.1
H-4	7.63 dd	7.62 dd	7.61 dd	7.59 dd
H-5	7.24 dd	7.24 dd	7.24 dd	7.22 dd
H-6	8.36 dd	8.35 dd	8.39 dd	8.35 dd
$J_{H\text{-}4,H\text{-}5}$	7.7	7.7	7.6	7.6
$J_{H\text{-}5,H\text{-}6}$	4.8	4.8	4.7	4.8
$J_{H\text{-}4,H\text{-}6}$	0.8	0.8	1.3	0.9
H-7	2.43 s	2.38 s	2.38 s	2.35 s

TABLE 8. : 25 MHz ^{13}C NMR data ($CDCl_3$) of the α-2',3'-O-isopropylidene derivatives (**9α**-**13α**).

	9α	10α	11α	12α	13α
C-1'	84.07	83.07	83.77	83.76	84.32
C-2'	82.73	81.91	82.55	82.57	82.85
C-3'	82.73	82.29	82.55	82.60	82.85
C-4'	84.98	83.77	84.78	84.82	84.80
C-5'	62.50	62.25	62.36	62.28	62.63
C-2	157.35	154.08	156.67	154.12	156.74
C-3	122.51	131.00	123.46	121.77	119.55
C-4	136.21	138.17	147.75	136.80	136.47
C-5	122.32	121.96	123.01	131.83	122.28
C-6	148.22	145.63	147.77	148.35	156.74
C-7	---	18.29	21.11	17.99	24.22
C-6'	112.58	112.59	112.42	112.46	112.49
C-7'	24.67	24.61	24.73	24.73	24.76
C-8'	26.07	26.03	26.13	26.13	26.12

lower field than the corresponding β-anomeric proton. To this rule few, if any, exceptions have been reported among C-nucleosides protected with a 2',3'-O-isopropylidene group.

However, in our case this chemical shift rule is violated by the anomeric pair **10α,β** with δH-1'α = 5.37 and δH-1'β = 5.42. With respect to the H-1' chemical shift of the other β-anomers in this series the H-1' of **10β** is shifted downfield approximately by 0.4 ppm ! This phenomenon is undoubtedly related to the ortho position of the pyridine methyl group with respect to the sugar moiety.

The H-2' chemical shift differences noticed in the α- and β-anomers are probably useful in determining the anomeric configuration of the 2',3'-O-isopropylidene

derivatives (**9** to **13**). Indeed, as can be seen from TABLES 3 and 4, δH-2'α appears 0.21 to 0.34 ppm downfield with respect to δH-2'β. This phenomenon also occurred in the pyridine C-nucleosides **4** to **8**, as well as in a series of pyridine C-nucleosides synthesized by Vrijens et al.[25]. In these compounds a ΔδH-2' of 0.29 to 0.42 was found. This "syn-upfield" effect for the H-2'β proton probably originates from the heterocyclic base.

Also an inversed Δδα/β-effect was observed for the H-3' resonances of **9** to **13**. An explanation for this phenomenon seems less obvious as no such effect was noticed for the parent nucleosides **4** to **8**. Therefore, it seems less advisable to use the ΔδH-3' data for the determination of the anomeric configuration of C-nucleosides or their 2',3'-O-isopropylidene derivatives.

The ^{13}C signals of the isopropylidene methyl groups are found at 25.3 ± 0.1 and 27.5 ± 0.1 ppm for the β-anomers and at 24.7 ± 0.1 and 26.1 ± 0.1 for the α-anomers (TABLES 8 and 9). This is comparable with the range of literature data[22] (25.5 ± 0.2 and 27.5 ± 0.2 ppm for the β-anomers and 24.9 ± 0.3 and 26.3 ± 0.2 ppm for the α-anomers).

In addition, the ^{13}C Δδ values for these two methyl groups are consistent with the chemical shift differences reported in literature[22] for analogous compounds (2.1 ± 0.1 and 1.4 ± 0.1 for the β- and α-anomers, respectively).

In conclusion, for 2',3'-O-isopropylidene protected pyridine C-nucleosides one should be careful when assigning the anomeric configuration with the aid of ^1H NMR. Perhaps in these cases one should rely on ^{13}C NMR data as well.For the assignment of the α- and β-configuration of 2-deoxy D-ribofuranosyl-C-nucleosides both the anomery at C-1' and the conformation of the sugar ring has to be taken into account. A study[26] shows that it is possible to discriminate between α and β C-nucleosides on the basis of their ^1H NMR spectra.

The spectra of the nucleosides **25α** and **25β** were recorded in CD_3OD and in DMSO-d_6 and are listed in TABLE 7 and TABLE 13. A conformational analysis was performed according to Altona's method[27]; the nucleoside is supposed to be

TABLE 9. : 25 MHz ^{13}C NMR data ($CDCl_3$) of the β-2',3'-O-isopropylidene derivatives (**9β** - **13β**). (t : tentative assignment)

	9β	10β	11β	12β	13β
C-1'	87.97	83.63	88.09	87.79	88.27
C-2'	86.75	86.83	86.87	86.78	87.03
C-3'	83.28	83.51	83.40	83.38	83.69
C-4'	86.87	86.89	86.87	86.78	87.18
C-5'	63.91	63.90	63.91	64.05	64.31
C-2	159.85	154.87	159.37	156.77	159.14^t
C-3	122.39^t	131.04	123.18^t	121.94	119.60
C-4	137.31	138.82	149.07	137.61	137.53
C-5	123.30^t	122.81	124.28^t	132.93	123.15
C-6	149.32	146.20	149.07	149.51	158.53^t
C-7	---	17.86	21.02	17.95	23.59
C-6'	113.19	112.74	113.37	112.75	112.65
C-7'	25.34	25.40	25.34	25.34	25.34
C-8'	27.47	27.47	27.47	27.47	27.47

in equilibrium between two extreme conformations N and S. The maximum torsion angles ϕ_N, ϕ_S, the pseudorotation angles P_N, P_S and the molar fractions X_N, X_S of the two conformers are allowed to vary in order to fit between calculated and observed coupling constants. According to the results of TABLE 14, the N conformation emerges for **25α** dissolved in DMSO-d_6 whereas the S conformation emerges in CD_3OD solution; for **25β**, the S conformation is the dominant species in the two solvents. The assignment of anomeric configuration was confirmed by a ^1H NMR NOE difference experiment[28]. Saturation of H-1' of **25β** resulted in a characteristic NOE effect on H-4'(2%), indicating a β-configuration.

TABLE 10. : 25 MHz ^{13}C NMR data (CD_3OD) of the 2-(2-deoxy-D-erythro-pento-1-enofuranosyl)-pyridine C-nucleosides (**15** - **19**).
(t : tentative assignment)

	15	16	17	18	19
C-1'	150.05	149.34	150.07^t	149.40	150.01
C-2'	103.26	106.08	102.98	102.24	102.79
C-3'	91.20	90.79	91.28	91.40	91.34
C-4'	76.39	76.66	76.54	76.72	76.66
C-5'	63.24	63.44	63.44	63.50	63.50
C-2	158.33	158.29	158.72	157.01	159.51^t
C-3	122.45^t	134.41	123.08^t	121.92	119.48
C-4	138.72	140.87	150.31^t	138.86	138.55
C-5	125.31^t	125.03	125.94^t	135.57	124.91
C-6	150.05	147.08	149.70	150.43	159.08^t
C-7	---	20.31	21.04	18.24	23.96

EXPERIMENTAL.

^1H NMR spectra and 2D NMR experiments were recorded at the University of Ghent (Belgium) using a Bruker WH-360 or a Bruker 500 MHz. The NOEDIFF mode of the Bruker software package was used for the NOE difference experiment. ^{13}C NMR spectra were recorded on a Jeol-JNM-FX-100 (25 MHz, RUCA, University of Antwerp (Belgium) connected to a TI-980 B computer system. The chemical shifts are expressed in parts per million with respect to tetramethylsilane.

DCI-mass spectra were run on a Ribermag-10-10B (Nermag S.A.) quadrupole mass spectrometer equipped with a SIDAR data system. Primary ionisation of the reagent gas (NH_3) was performed by 70 eV electrons. The ionisation current was 0.08 mA and the pressure in the ion source was 0.1 mmHg. Analytical TLC was performed on silica plates (Kieselgel 60 F_{254} Merck, Darmstadt, 0.25 mm). Preparative centrifugal circular thin layer chromatography (CCTLC) was carried

TABLE 11. : 25 MHz ^{13}C NMR data ($CDCl_3$) of the furan compounds (**20** - **24**). (t : tentative assignment).

	20	21	22	23	24
C-1'	149.20	146.39	148.89^t	151.87	148.71
C-2'	110.08^t	111.42^t	109.96^t	108.86^t	109.84^t
C-3'	109.47^t	108.01^t	109.41^t	107.89^t	109.35^t
C-4'	155.53	154.55	155.16	154.13	155.22^t
C-5'	57.39	56.11	57.51	56.35	57.39
C-2	153.15	150.96	153.03	153.88	153.15^t
C-3	118.61	128.91	119.53^t	117.45	115.93
C-4	136.64	138.47	148.04^t	136.46	136.95
C-5	121.90	120.47	123.00^t	130.67	121.66
C-6	149.50	145.42	149.07	148.52	158.33
C-7	---	19.62	21.14	17.24	24.37

TABLE 12. : 25 MHz ^{13}C NMR data of the 2-(2-deoxy-D-ribofuranosyl)-3-methylpyridine C-nucleosides (**25**α,β).

	25α[a]	25β[a]	25β[b]
C-1'	87.08	87.75	89.45
C-2'	38.22	DMSO-d_6	42.30
C-3'	76.54	76.60	77.82
C-4'	72.03	72.28	74.16
C-5'	61.92	62.47	63.80
C-2	158.11	157.81	159.39
C-3	131.55	131.49	132.70
C-4	138.62	138.25	140.20
C-5	122.78	122.72	124.18
C-6	145.93	145.99	147.20
C-7	17.81	17.57	17.99

a : recorded in DMSO-d_6; b : recorded in CD_3OD.

TABLE 13. : Observed (first column) and calculated (second column) coupling constants (J). (a : recorded in CD_3OD; b : recorded in DMSO-d_6)

Compound	$J_{H-1',H-2'\beta}$		$J_{H-1',H-2'\alpha}$		$J_{H-2'\beta,H-3'}$		$J_{H-2'\alpha,H-3'}$		$J_{H-3',H-4'}$	
25α[a]	7.92	8.07	4.98	4.92	6.54	6.45	3.88	4.18	3.40	3.14
25β[a]	9.27	9.32	6.25	6.28	6.03	6.08	2.38	2.46	2.38	2.33
25α[b]	7.10	6.47	6.87	6.14	7.10	7.49	5.50	6.09	5.50	5.29
25β[b]	8.97	9.06	6.35	6.36	5.86	5.88	2.55	2.69	2.55	2.48

TABLE 14. : Conformational parameters of 25α and 25β.
(a : recorded in CD_3OD; b : recorded in DMSO-d_6)

Compound	X_N	P_N	ϕ_N	P_S	ϕ_S	RMS
25α[a]	0.32	-12.31	43.84	-131.30	43.40	0.195
25β[a]	0.15	2.00	35.00	163.19	34.16	0.053
25α[b]	0.66	-17.92	30.66	131.01	47.26	0.540
25β[b]	0.15	5.93	47.45	165.69	33.06	0.081

out on a Chromatotron® (Harrison Research, Palo Alto, CA). Stationary phase: Kieselgel 60 PF_{254} gipshaltig, Merck, Darmstadt, layer thickness 2 mm, flow rate 6 ml/min, unless stated otherwise. Purities were ascertained by HPLC analyses on a Hewlett-Packard HP-1084 apparatus (column : Lichrosorb 10RP8 (25 cm x 4,6 mm)). Reactions involving organometallic reagents were performed in oven-dried glassware under a N_2 atmosphere. 1,2-Dimethoxyethane and diethylether were dried by distillation from sodium/benzophenone ketyl prior to use. The cytostatic and antiviral activity assays were carried out according to previously published procedures[29-32].

2-(2,3-O-isopropylidene-α-D-ribofuranosyl)pyridines (9α - 12α)

p-Toluenesulphonic acid (0.23 g = 1.2 eq) was added to a stirred solution of 1 mmol 4α, 5α, 6α or 7α[13] in 20 ml acetone and 1 ml 2,2-dimethoxypropane. Stirring was continued for 3 hours. After neutralization with 1N $NaOCH_3$ the reaction mixture was evaporated. The residue was then triturated with hot chloroform and filtered. The filtrate was evaporated leaving a yellow oil that was purified by means of CCTLC (eluant: CH_2Cl_2/MeOH 99/1 to 95/5) to yield a pale yellow oil.

9α : 80%; Rf : 0.34 (CH_2Cl_2/MeOH 9/1); DCI-MS : 252 (100%) [MH^+]

10α : 82%; Rf : 0.49 (CH_2Cl_2/MeOH 9/1); DCI-MS : 266 (100%) [MH^+]

11α : 83%; Rf : 0.36 (CH_2Cl_2/MeOH 9/1); DCI-MS : 266 (100%) [MH^+]

12α : 83%; Rf : 0.39 (CH_2Cl_2/MeOH 9/1); DCI-MS : 266 (100%) [MH^+]

2-(2,3-O-isopropylidene-β-D-ribofuranosyl)pyridines (9β - 12β)

The same procedure as for the α-anomers was used, only this time the reaction time was 90 minutes.

9β : 85%; Rf : 0.79 (CH_2Cl_2/MeOH 9/1); DCI-MS : 252 (100%) [MH^+]

10β : 92%; Rf : 0.75 (CH_2Cl_2/MeOH 9/1); DCI-MS : 266 (100%) [MH^+]

11β : 85%; Rf : 0.75 (CH_2Cl_2/MeOH 9/1); DCI-MS : 266 (100%) [MH^+]

12β : 87%; Rf : 0.68 (CH_2Cl_2/MeOH 9/1); DCI-MS : 266 (100%) [MH^+]

2-(2,3-O-isopropylidene-α-D-ribofuranosyl)-6-methylpyridine (13α) and 2-[2,3-O-isopropylidene-5-O-(2'-methoxyisopropyl)-α-D-ribofuranosyl)-6-methylpyridine (14α). p-Toluenesulphonic acid (0.34 g = 1.2 eq) was added to a stirred solution of 400 mg 2-(α-D-ribofuranosyl)-6-methylpyridine (**8α**)[13] in 40 ml 2,2-dimethoxypropane. Stirring was continued for 12 hours. After neutralization with 1N $NaOCH_3$ the reaction mixture was evaporated. The residue was then triturated with hot chloroform (50 ml) and filtered. The filtrate was evaporated leaving a yellow oil. Chromatography on silica (Kieselgel 60, 230-400 mesh, 20 cm x 15 mm I.D., EtAc/Hexane 1/1) yielded pale yellow oils of **13α** and **14α**.

13α : 42%; Rf : 0.37 (EtAc); DCI-MS : 266 (100%) [MH^+]

14α : 45%; Rf : 0.59 (EtAc); DCI-MS : 338 (100%) [MH^+], 306 (100%) [MH^+-CH_2O], 266 (50%) [MH^+-CH_2=$C(OCH_3)(CH_3)$]; ^{1}H-NMR ($CDCl_3$) δ : 1.36 (6 H, s, $CH_3OC(C\underline{H}_3)_2O$-), 1.28 + 1.39 (6 H, 2s, -$OC(C\underline{H}_3)_2O$-), 2.51 (3 H, s, pyr CH_3), 3.21 (3 H, s, $C\underline{H}_3O$-), 3.56 + 3.57 (2 H, dd, H-5'+ H-5"), 4.41 (1 H, t, H-4', $J_{H-4',H-5'}$ = 4.9 Hz, $J_{H-4',H-5"}$ = 4.6 Hz), 4.87 (1 H, dd, H-3', $J_{H-3',H-4'}$ = 0.9 Hz), 5.05 (1 H, dd, H-2', $J_{H-2',H-3'}$ = 5.9 Hz), 5.21 (1 H, d, H-1', $J_{H-1',H-2'}$ = 4.1 Hz), 7.04 (1 H, d, H-3, $J_{H-3,H-4}$ = 7.6 Hz), 7.36 (1 H, d, H-5), 7.58 (1 H, t, H-4, $J_{H-4,H-5}$ = 7.9 Hz); ^{13}C-NMR ($CDCl_3$) δ : 24.42 + 24.86 + 26.17 (pyr $\underline{C}H_3$ + other $\underline{C}H_3$'s), 48.64 ($\underline{C}H_3O$), 61.46 (C-5'), 82.90 + 83.40 + 85.10 (2x) (C-1' - C-4'), 100.25 ($CH_3O\underline{C}(CH_3)_2O$-), 112.49 (-$O\underline{C}(CH_3)_2O$-), 119.31 (C-3), 122.04 (C-5), 136.12 (C-4), 156.98 (C-2 + C-6).

2-(2,3-O-isopropylidene-β-D-ribofuranosyl)-6-methylpyridine (13β) and 2-[2,3-O-isopropylidene-5-O-(2'-methoxyisopropyl)-β-D-ribofuranosyl)-6-methylpyridine (14β). The same procedure was used as described for compounds 13α and 14α.

13β : 79%; Rf : 0.66 (EtAc); DCI-MS : 266 (100%) [MH^+]

14β : 12%; Rf : 0.81 (EtAc); DCI-MS : 338 (100%) [MH^+], 306 (58%) [MH^+-CH_2O], 266 (100%) [MH^+-CH_2=$C(OCH_3)(CH_3)$]; ^{1}H-NMR ($CDCl_3$) δ : 1.28 + 1.31 (6 H, 2s, $CH_3OC(C\underline{H}_3)_2O$-), 1.39 + 1.62 (6 H, 2s, -$OC(C\underline{H}_3)_2O$-), 2.51 (3 H, s, pyr CH_3), 3.20 (3 H, s, CH_3O-), 3.54 (1 H, dd, H-5"), 3.59 (1 H, dd, H-5', $J_{H-5',H-5"}$ = -10.2 Hz), 4.30 (1 H, m, H-4', $J_{H-4',H-5'}$ = 4.4 Hz, $J_{H-4',H-5"}$ = 5.6 Hz), 4.66 (1 H, dd, H-3', $J_{H-3',H-4'}$ = 4.0 Hz), 4.96 (1 H, dd, H-2', $J_{H-2',H-3'}$ = 6.6 Hz), 5.06 (1 H, d, H-1', $J_{H-1',H-2'}$ = 3.6 Hz), 7.03 (1 H, d, H-3, $J_{H-3,H-4}$ = 7.7 Hz), 7.27 (1 H, d, H-5), 7.53 (1 H, t, H-4, $J_{H-4,H-5}$ = 7.8); ^{13}C-NMR ($CDCl_3$) δ : 24.27 + 24.47 + 25.64 + 27.49 (pyr CH_3 + other CH_3's), 48.54 ($\underline{C}H_3O$-), 61.60 (C-5'), 82.51 + 84.46 + 85.73 + 87.03 (C-1' - C-4'), 100.06 ($CH_3O\underline{C}(CH_3)_2O$-), 113.90 (-$O\underline{C}(CH_3)_2O$-), 117.90 (C-3), 122.04 (C-5), 136.46 (C-4), 158.88 + 157.91 (C-2 + C-6).

2-(2-deoxy-D-erythro-pent-1-enofuranosyl)pyridines (15-19) and 2-(5-hydroxymethylfur-2-yl)pyridines (20-24) with the aid of phenyl lithium.

To a stirred solution of 0.38 mmol 9 - 13 in 20 ml ether was added through a septum 0.63 ml (3.3 eq) phenyl lithium (2.0 M in cyclohexane/ether 70/30). After 15 min the solution was poured onto 50 ml H_2O/CH_3OH (9/1). This mixture was carefully acidified by addition of 0.1 N HCl to pH = 7.5, evaporated to dryness and chromatographed by means of CCTLC (eluant CH_2Cl_2/CH_3OH 93/7).

15 : (from **9α**) : 89%, (from **9β**) : 93%; Rf : 0.34 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 194 (100%) [MH^+], 176 (11%) [MH^+-H_2O]; UV λ_{max} (CH_3OH) 290 (ε : 5500). Anal. Calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found : C, 62.25; H, 5.88; N, 7.13.

20 : 2%; Rf : 0.48 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 176 (100%) [MH^+]; UV λ_{max} (CH_3OH) 305 (ε : 6800), 275 (ε : 5500).

16 : (from **10α**) : 90%, (from **10β**) : 88%; Rf : 0.32 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 208 (100%) [MH^+], 190 (49%) [MH^+-H_2O], 160 (17%) [$MH^+-H_2O-CH_2O$]; UV λ_{max} (CH_3OH) 285 (ε : 4800). Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found : C, 63.83; H, 6.16; N, 6.68.

21 : 1 - 2%; Rf : 0.53 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 190 (100%) [MH^+]; UV λ_{max} (CH_3OH) 305 (ε : 5500), 270 (ε : 5100).

17 : (from **11α**) : 85%, (from **11β**) : 82%; Rf : 0.27 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 208 (100%) [MH^+], 190 (57%) [MH^+-H_2O], 160 (27%) [$MH^+-H_2O-CH_2O$]; UV λ_{max} (CH_3OH) 290 (ε : 7200). Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found : C, 63.86; H, 6.22; N, 6.66.

22 : 1 - 2%; Rf : 0.48 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 190 (100%) [MH^+]; UV λ_{max} (CH_3OH) 305 (ε : 7100), 275 (ε : 5700).

18 : (from **12α**) : 95%, (from **12β**) : 92%; Rf : 0.32 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 208 (100%) [MH^+], 190 (25%) [MH^+-H_2O], 160 (25%) [$MH^+-H_2O-CH_2O$]; UV λ_{max} (CH_3OH) 292 (ε : 7600), 272 (ε : 7800). Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found : C, 63.73; H, 6.40; N, 6.73.

23 : 1 - 2%; Rf : 0.48 (CH_2Cl_2/CH_3OH 9/1); DCI-MS : 190 (100%) [MH^+];

UV λ_{max} (CH_3OH) 305 (ε : 6900), 270 (ε : 5500).

19 : (from **13α**) : 87%, (from **13β**) : 84%; Rf : 0.25 (EtAc); DCI-MS : 208 (100%) [MH^+], 190 (100%) [MH^+-H_2O], 160 (5%) [MH^+-H_2O-CH_2O]; UV λ_{max} (CH_3OH) 295 (ε : 6100). Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found : C, 63.97; H, 6.42; N, 6.48.

24 : 1 - 3%; Rf : 0.75 (EtAc); DCI-MS : 190 (100%) [MH^+]; UV λ_{max} (CH_3OH) 305 (ε : 6200), 270 (ε : 5200).

2-(2-deoxy-D-erythro-pent-1-enofuranosyl)-6-methylpyridine (19) with the aid of lithium tetramethylpiperidide (LTMP). 0.30 ml (1.8 mmol) Tetramethylpiperidine was dissolved in 30 ml DME and placed in a 3-necked flask equipped with a magnetic stirrer, a N_2-inlet tube, a dropping funnel and a $CaCl_2$-tube. The solution was cooled to -78°C in an acetone/dry ice bath. Then 1.13 ml (1.8 mmol) BuLi (1.6M in hexane) precooled at -20°C was added. Stirring was continued for 60 minutes. A solution of 160 mg (0.6 mmol) **13α** or **13β** in 10 ml DME was added slowly (15 min). Cooling was continued for 2 hours whereafter the mixture was allowed to warm up overnight (room temperature). Then the solution was poured onto 50 ml H_2O/CH_3OH (9/1). This mixture was carefully acidified by addition of 0.1 N HCl to pH = 7.5, evaporated to dryness and chromatographed by means of CCTLC (eluant CH_2Cl_2/MeOH 93/7).

From **13α** : yield **19** : 18% ; **24** : 1%; recuperated **13α** : 70%

From **13β** : yield **19** : 16% ; **24** : 2%; recuperated **13β** : 74%

2-(2-deoxy-D-erythro-pent-1-enofuranosyl)pyridine (15) with the aid of lithium tetramethylpiperidide (LTMP). Essentially the same procedure was performed with 4.5 eq LTMP on 100 mg **9β** as described for **19**. The eluant for CCTLC was changed gradually from 98/2 to 90/10 during elution.

Yields: **15**: 36%; **20**: 6%

Recuperated **17**: 45%

2-(2-deoxy-D-ribofuranosyl)-3-methylpyridine (25α,β)

A mixture of 30 mg **16**, 15 mg Pd/C (10%) and 20 ml EtOH under H_2 at 1 atm was vigourously stirred for 15 min, filtered using a Celite pad and further purified for chromatography on a Sep-pak silica cartridge (Millipore®, Waters Ass.). CCTLC (plate thickness : 1.5 mm, flow rate : 5 ml/min, eluant for plate conditioning : CH_2Cl_2/MeOH 98/2, the eluting power was increased when the bands were slightly separated : 98/2 to 95/5) yielded two transparant congealed oils (**25**) and a yellow oil (**21**).

25α : 25%; Rf: 0.42 (CH_2Cl_2/EtOH 9/1) DCI-MS : 210 (100%) [MH^+]

25β : 61%; Rf: 0.39 (CH_2Cl_2/EtOH 9/1) DCI-MS : 210 (100%) [MH^+]

21 : 8%

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