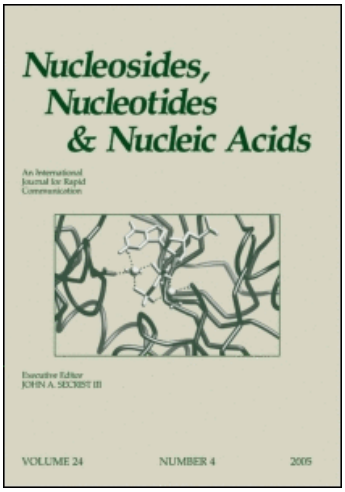

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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M. Belmans[a]; I. Vrijens[a]; E. Esmans[a]; R. Dommisse[a]; J. Lepoivre[a]; F. Alderweireldt[a]; L. Townsend[a]; L. Wotring[a]; J. Balzarini[b]; E. De Clercq[c]
[a] University of Antwerp (RUCA), Laboratory for Organic Chemistry, Antwerp, Belgium [b] Department of Medicinal Chemistry, College of Pharmacy and Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA [c] 2 Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

To cite this Article Belmans, M. , Vrijens, I. , Esmans, E. , Dommisse, R. , Lepoivre, J. , Alderweireldt, F. , Townsend, L. , Wotring, L. , Balzarini, J. and De Clercq, E.(1986) 'Synthests and Biological Evaluation of a Series of Substituted-2-Pyridine-C-Nucleosides. Part II', Nucleosides, Nucleotides and Nucleic Acids, 5: 4, 441 — 455
To link to this Article: DOI: 10.1080/07328318608068685
URL: http://dx.doi.org/10.1080/07328318608068685

NUCLEOSIDES & NUCLEOTIDES, 5(4), 441-455 (1986)

SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF SUBSTITUTED-2-PYRIDINE-C-NUCLEOSIDES. Part II

M. Belmans°, I. Vrijens, E. Esmans, R. Dommisse, J. Lepoivre, F. Alderweireldt, L. Townsend[1], L. Wotring[1], J. Balzarini[2], E. De Clercq[2], University of Antwerp (RUCA), Laboratory for Organic Chemistry, Groenenborgerlaan 171, B 2020 Antwerp, Belgium

1 Department of Medicinal Chemistry, College of Pharmacy and Department of Chemistry, University of Michigan, Ann Arbor, Michigan USA

2 Rega Institute for Medical Research, University of Leuven, B 3000 Leuven, Belgium.

ABSTRACT :

A cyclisation reaction of the D-allo- and D-altro isomers of 2-(2,4:3,5'-di-O-benzylidenepentitol-1-yl)pyridine derivatives to afford the corresponding substituted 2-(D-ribofuranosyl)pyridine-C-nucleosides, was investigated. The latter compounds were obtained in good yields (90%) if the reaction was performed in 1 N HCl. The structures were confirmed by ^{13}C-NMR studies and an HPLC method which was developed specifically for a determination of their purity. All compounds were evaluated for biological activity in a variety of antiviral and antitumor cell systems in vitro.

1. INTRODUCTION.

The isolation and characterization of several naturally occuring C-nucleosides[1] (e.g. formycin, formycin B, pseudouridine, showdomycin, etc) was followed by reports[2] on their

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biological and chemotherapeutic properties. This has prompted[3] the synthesis of a wide range of structurally related C-nucleosides. In general the cytostatic and/or antiviral activity of a nucleoside is related to its inhibition of certain enzymes and/or its incorporation into DNA/RNA.[4]

The oncolytic C-nucleoside 2-(β-D-ribofuranosyl)thiazole-4-carboxamide (tiazofurin) has emerged as a promising new drug with activity being reported against a number of tumors. It has been shown that tiazofurin has a different mechanism and mode of action since it acts *via* the formation of an NAD^+-analogue[3,4] in which the nicotinamide nucleoside moiety has been replaced by tiazofurin. This prompted us to initiate a program involving the synthesis of pyridine-C-nucleosides designed as nicotinamide nucleoside analogs. The synthesis of some appropriate precursors, e.g. the D-allo- and D-altro- isomers of 2-(1-D-methylsulphonyl-2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine derivatives, using organometallic lithiopyridines as intermediates has been previously reported[5] from our laboratory.

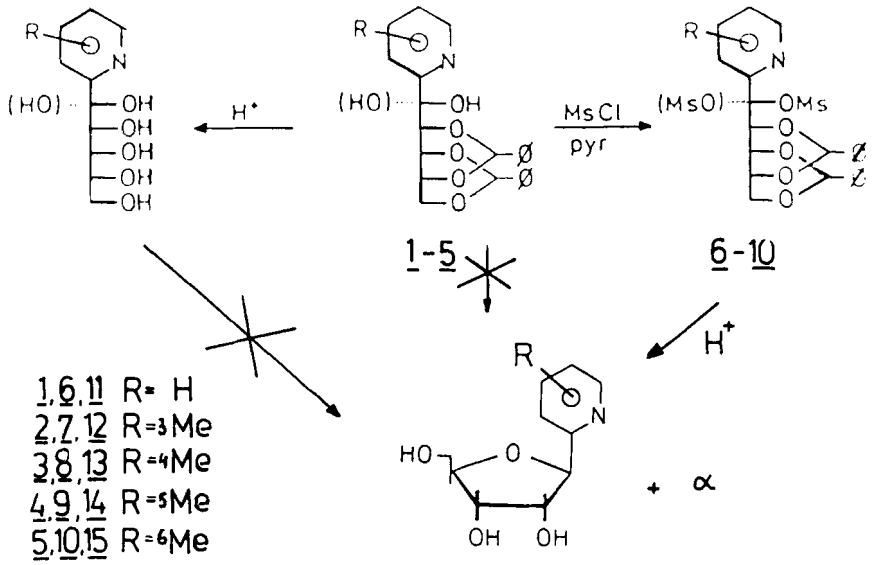
We now wish to report on the formation and biological evaluation of a new series of pyridine-C-nucleosides.

2. RESULTS AND DISCUSSION.

2.1. SYNTHESIS

We have previously reported that a direct cyclization of the D-allo- D-altro isomers of 2-(2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine derivatives (1-5) in an acidic medium was unsuccessful. In an effort to circumvent this problem, a mesylate function was introduced at the C_1' position of compounds 1-5 to afford 6-10.

In our first attempt to obtain the corresponding ribofuranosyl compounds 11-15, the D-allo- and D-altro-isomers of 2-((1-D-methylsulfonyl)-2,4:3,5-di-O-benzylidene-pentitol-1-yl)pyridine derivatives (6-10) were treated with 0,05 M HCl/dioxane (1/100). As shown by Buchanan et al.,[6] these



SCHEME 1

reaction conditions were appropriate for a cyclization reaction of the analogous mesyl compounds in the pyrazole series, however, the pyridine-C-nucleosides 11-15 were obtained in low yields (15%) under these conditions. Therefore, a study designed to optimize the conditions required for the cyclization reaction was undertaken using 1 N HCl, without the addition of dioxane.

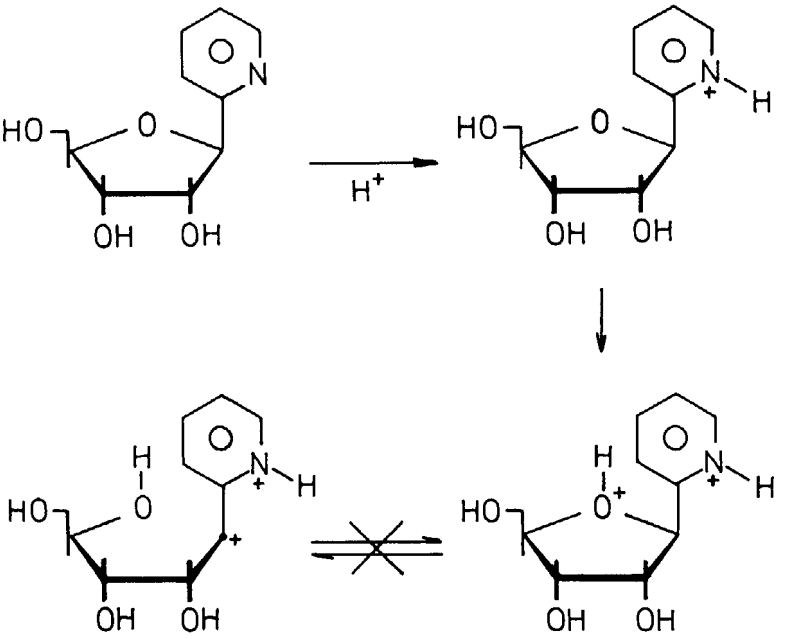
Aliquots were taken at different time intervals and analyzed by HPLC on a μ-Bondapack C18 column using H_2O/CH_3OH (90/10) as the eluant. (UV-detector 254 nm/flow rate 1 cc/min). The results for compound 11 are summarized in Table 1 and clearly show that the cyclization reaction is complete after 50 minutes.

Analysis of the reaction products 11 to 15 by C^{13} NMR (see section on C^{13} NMR) showed that no pyranosyl compounds were formed during the cyclization procedure described above. This is of considerable interest since it has been previously observed that β-pseudouridine (φ), when dissolved

Table 1

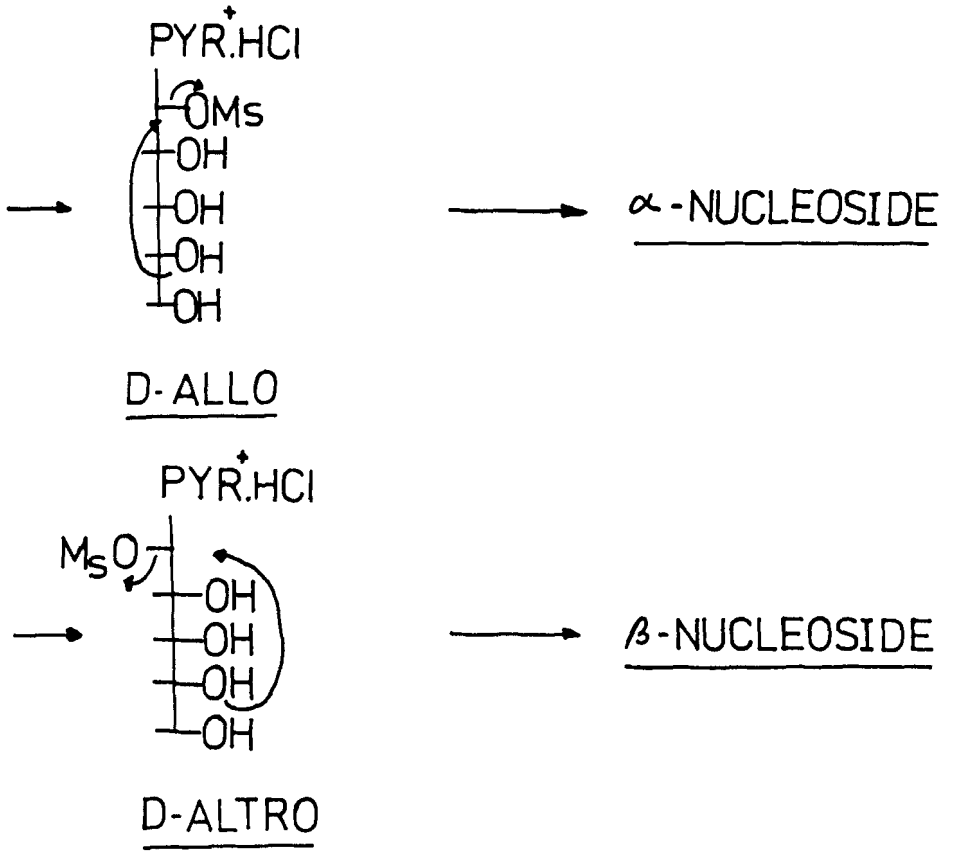
reaction time (min)	total yield ($\alpha + \beta$)
4'	73.0%
6'	79.5%
9'	80.0%
13'	87.0%
18'	89.0%
24'	91.0%
48'	100.0%

in an acidic solution, forms an equilibrium mixture of both α,β-ribofuranose- and α,β-ribopyranose isomers. This phenomenon was not observed in the series of 2-(D-ribofuranosyl)pyridine derivatives (11-15). This stability can be explained by the initial protonation of the basic pyridine ring nitrogen atom which opposes the intermediate generation of a carbonium ion at C_1' which is necessary for an equilibration to occur :



SCHEME 2

SCHEME 3



The formation of both α- and β-ribofuranose-pyridine-C-nucleosides during the cyclization procedure (6-10->11-15) is probably due to an SN2-like ring closure mechanism starting from both the D-allo- and D-altro- isomers as illustrated in Scheme 3.

2.2. PURIFICATION AND HPLC-ANALYSIS.

The initial purification of this reaction mixture was accomplished by extracting the aqueous solution with chloroform to remove all of the benzaldehyde which had formed during the cyclisation reaction. The methane sulphonic acid salt was then separated from the 2-(D-ribofuranosyl)pyridine derivatives on an Affigel 601 (Biorad) column using 0.25 M

NH_4OAc (pH = 8.8). The cis-diol nucleosidic fraction was then eluted with 0.1 N formic acid and collected. The solution was adjusted to pH 7 using dilute ammonia and lyophylized. The pyridine-C-nucleosides were isolated in 90% yield.

In order to check the purity of these compounds, prior to biological evaluation, an HPLC analysis was accomplished on a μ-Bondapack-C18-column using water/methanol (85/15) as the eluant, at a flow-rate of 1mL/min. The results of this investigation are summarized in Table 2

It is of some interest to note that under these conditions both the α- and β-isomers were separated. The β-isomer was assigned to the peak eluting with the largest k-value and this assignment was supported by an analysis of the ^{13}C-NMR spectra of the anomeric mixture (See ^{13}C NMR). Since C_1' of the β- anomer resonates at a lower field than the corresponding C_1' of the α anomer, integration of these signals provides an approximation of the anomeric composition which in turn can be compared with the data obtained from the HPLC-analysis (Table 3). Furthermore these results were supported by on-line DLI/LCMS[7] and HPLC-literature data.

2.3. ^{13}C-NMR.

The ^{13}C NMR spectra of the pyridine-C-nucleosides (11 to 15) were recorded in D_2O using DMSO as an internal standard. The chemical shift values were converted to the TMS scale using $\delta DMSO = \delta TMS + 39.5$ ppm. From the results, depicted in Figure 1, it can be seen each carbon atom is present in duplicate with different intensity. This can be explained by the presence of α,β-anomeric mixture. The pyridine C-atoms are well separated from the resonance signals attributed to the sugar moiety and resonate between 140 and 157 ppm. The assignment of these signals to specific carbon atoms was achieved by chemical shift arguments in the case of 2-(D-ribofuranosyl)-pyridine 11.

Table 2 Retention Time in Minutes

	α	β
11	4.52	5.25
12	5.07	10.74
13	7.92	11.83
14	8.48	12.26
15	6.83	12.42

Table 3

	α	β
11	43%	57%
12	46%	54%
13	46%	54%
14	44%	56%
15	45%	55%

For compounds 12-15, an analogous procedure was followed while taking into account the substituent shift increments for the introduction of a methyl group at the different ring positions. This increment is most pronounced on the carbon atom where the methyl function resides and the results are presented in Table 4.

In regards to the different sugar atoms (Table 5), the signal located in the 59 to 63 ppm region was assigned to the primary C_5'-carbon atom. This is the expected value for this C-atom. According to the data recently published by Beier[12], it was possible to assign C_2' and C_3'. The differences in intensity between the signals of the α- and β-isomers can be used as an additional assignment tool when the signals are close to each other. The C_1' and C_4'-atoms resonate close to each other. Their assignment was done by

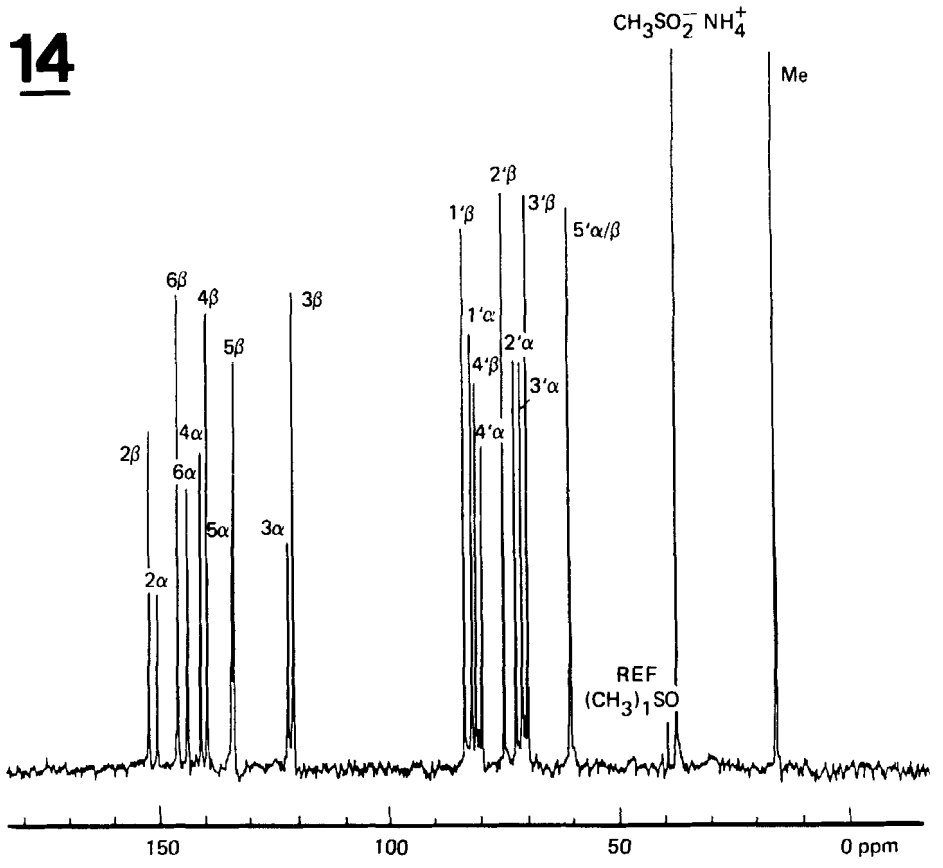


Figure 1

selective decoupling experiments of the H_1'and H_4'-protons ; which clearly showed that the peak at the lowest field corresponds to C_1'-carbon atom. It is also clear that no pyranosyl type-C-nucleosides were formed during the cyclisation reaction, since the C_5' signals occur at values which are usually observed for primary alcohols.

2.4. BIOLOGICAL STUDIES. IN VITRO ANTITUMOR EVALUATION.

The antiviral and antitumor effects of compounds 11 to 15 (α,βmixtures purified by chromatography) were evaluated in a variety of assay systems (table 6). Under conditions were the proper controls showed the usual antiviral activity

Table 4

	2	3	4	5	6	Me
11	β 156.78	125.69	141.96	123.55	147.28	/
	α 155.03	125.79	143.28	124.76	145.38	/
12	β 151.96	132.77	142.03	123.39	145.01	15.99
	α 149.64	133.87	138.80	124.18	141.54	15.62
13	β 156.16	122.41	154.15	124.85	143.31	20.07
	α 153.48	123.69	151.47	124.73	141.11	20.43
14	β 152.20	120.77	135.35	133.62	145.93	16.35
	α 150.37	121.80	140.81	133.93	143.73	
15	β 152.93	120.71	145.68	126.15	152.69	18.36
	α 151.23	121.56	145.20	125.95	150.43	

(ribavirin, carbocyclic 3-deazaadenosine) or antitumor activity (5-fluoro-2'-deoxyuridine), none of the test compounds exhibited an appreciable antiviral or antitumor effect in any of the assay systems (table 6).

It seems probable that these compounds were not metabolized to analogues of NAD^+, since they lack the amide substituent. Alternatively, if the NAD^+-analogues were formed, they apparently did not a inhibit any critical cellular enzymes. The synthesis of 2-pyridine-C-nucleosides with an amide substituent is under investigation.

3. EXPERIMENTAL

3.1. METHODS

^{13}C-NMR spectra were recorded on a JEOL FX-100 connected to a TI-980 B computer system. DCI-mass spectra were run on a RIBERMAG 10-10B (NERMAG SA) quadrupole mass-spectrometer equipped with a SIDAR data system. Primary ioniza-

Table 5

		C_4'	C_2'	C_3'	C_1'	C_5'*
11	β	83.63	76.90	71.79	85.58	62.58
	α	81.63	74.32	73.00	83.19	62.43
12	β	79.22	74.28	68.92	82.63	60.40
	α	72.27	70.63	79.78	80.56	59.73
13	β	80.93	74.96	69.78	83.73	60.46
	α	78.98	72.40	70.93	81.17	
14	β	81.05	74.96	69.78	83.42	60.58
	α	79.77	72.28	71.06	81.90	60.46
15	β	79.04	75.26	69.17	83.48	60.33
	α	77.88	72.33	70.81	81.47	59.72

* may be exchanged

tion of the reagent gas (NH3) was performed by 70 eV electrons using an emission current of 0.08mA. The ion-source pressure was around 0.3 Torr. Elemental analyses were done at JANSSEN PHARMACEUTICA, (Beerse Belgium). The 360 MHz ^{1}H-NMR spectra were taken at the University of Ghent by G. Verhegge. ^{1}H-NMR data are listed in Tables 7 and 8.

3.2. SYNTHESIS

The 1-(1-O-methylsulphonyl-2,4:3,5-di-O-benzylidene-D-ribopentahydroxypentyl)pyridine (500 mg) was stirred in a 1 N HCl-solution (20 ml) and heated for 50 minutes at reflux temperature. The reaction was quenched by cooling the

Table 6

Biological evaluation for antiviral and antitumor cell activity

Assay system*	Minimal inhibitory concentration† (μg/ml)		
	Compounds 11 through 15	Reference compounds [9,10,11]	
Antiviral activity		Ribavirin	Carbocyclic 3-deazaadenosine
HSV-1/PRK	> 400	> 400	> 400
HSV-2/PRK	> 400	> 400	> 400
VV/PRK	> 400	2	2
VSV/PRK	> 400	100	0.2
VSV/HeLa	> 400	7	1
Coxsackie-B4/HeLa	> 400	40	> 400
Polio-1/HeLa	> 400	70	> 400
Parainfluenza-3/Vero	> 400	70	7
Reo-1/Vero	> 400	150	10
Sindbis/Vero	> 400	70	> 400
Coxsackie-B4/Vero	> 400	> 400	7
SFV/Vero	> 400	150	> 400
Rhino-1A/WI-38	> 400	150	> 400
Rhino-9/WI-38	> 400	70	> 400
Antitumor cell activity		5-Fluoro-2'-deoxyuridine	
L1210	> 1000	0.0003	
FM3A	> 1000	0.002	
Raji	> 1000‡	0.005	
Molt/4F	> 1000	0.04	

*Abbreviations : HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VV, vaccinia virus; VSV, vesicular stomatitis virus; SFV, Semliki Forest virus; PRK, primary rabbit kidney; HeLa, continuous line of human epithelioma cells; Vero, continuous line of African green monkey kidney cells; WI-38, human diploid fibroblast cells; L1210, murine leukemia cells; FM3A, murine mammary carcinoma cells; Raji, human B-lymphoblast cells; Molt/4F, human T-lymphoblast cells.

†Required to reduce virus-induced cytopathogenicity or tumor cell growth by 50 %.

‡For compounds 11 α and 15 α/β the values were 675 and 803 μg/ml, respectively.

Table 7

360 MHz-^{1}H-NMR-data(1)

H_1'	H_2'	H_3'	H_4'	H_5'	H_5''
5.12 (d,3.4)	4.37 (t,3.9)	4.31 (dd,4.4)	4.10 (m)	3.87 (dd,2.70)	3.68 (dd,4.55
4.85 (d,5.5)	4.13 (t,5.1)	4.08 (m)	4.05 (m)	3.88 (dd,3.00)	3.72 (dd,4.10
5.39 (d,4.1)	4.52 (t,3.85)	4.26 (dd,4.35)	4.13 (m)	3.85 (dd,2.60)	3.71 (dd,3.90
5.18 (d,4.7)	4.26 (t,5.0)	4.16 (m)	4.08 (m)	3.96 (dd,2.80)	3.72 (dd,2.45
5.10 (d,3.4)	4.36 (t,4.35)	4.30 (dd)	4.10 (m)	3.86 (dd,2.65)	3.67 (dd,4.60
4.79 (d,5.6)	4.12 (t,5.10)	4.04 (m)	4.02 (m)	3.87 (dd,2.85)	3.72 (dd,3.40
5.09 (d,3.4)	4.35 (t,3.9)	4.29 (dd,4.4)	4.10 (m)	3.85 (dd,2.65)	3.67 (dd,4.60
4.79 (d,5.6)	4.12 (t,51)	4.04 (m)	4.02 (m)	3.86 (dd,2.90)	3.71 (dd,3.60
5.09 (d,3.6)	4.39 (t,3.95)	4.29 (dd,4.35)	4.10 (m)	3.85 (dd,2.75)	3.67 (dd,4.65
4.82 (d,5.1)	4.16 (t,5.0)	4.09 (m)	4.05 (m)	3.90 (dd,2.85)	3.72 (dd,3.35

(1): taken in CD_3OD.

Table 8 : pyridine protons

11	α	7.60(d)	7.82(t)	7.29(dd)	8.45(d)	/
11	β	7.56(d)	7.82(t)	7.30(dd)	8.47(d)	
12	α	/	7.70(d)	7.30(m)	8.38(d)	2.40(s)
12	β	/	7.63(d)	7.25(m)	8.35(d)	2.40(s)
13	α	7.40(s)	/	7.15(d)	8.34(d)	2.38(s)
13	β	7.45(s)	/	7.18(d)	8.31(d)	2.38(s)
14	α	7.50(d)	7.65(d)	/	8.30(s)	2.34(s)
14	β	7.46(d)	7.64(d)	/	8.33(s)	2.32(s)
15	α	7.18(d)	7.71(t)	7.40(d)	/	2.50(s)
15	β	7.20(d)	7.70(t)	7.32(d)	/	2.50(s)

solution in an ice bath. The benzaldehyde was removed by extraction with chloroform (3 x 20 ml). Analysis of these chloroform extracts showed that the only compound present was benzaldehyde and the absence of unreacted mesylate starting material or other side products. The water layer was evaporated to dryness and after the addition of water (10 mL), the pH was adjusted to 7 with dilute ammonia. The water layer was again extracted with chloroform (3 x 20 mL) and applied to an Affigel 601 column, using 0.25 M NH_4OAC (pH 8.8). The nucleosides were isolated by elution with formic acid (0.1 N) and the residual ammonium formate was removed by lyophilisation. The other nucleosides were prepared in the same way. The α- and β-isomers were not separated on a large scale prior to the biological studies.

Elemental Analysis

11	Calc:	56.87 %C	6.16 %H	6.63 %N	
	Found:	56.75 %C	6.13 %H	6.60 %N	yield : 90%

12-15	Calc:	58.67 %C	6.67 %H	6.22 %N	
12		58.82 %C	6.70 %H	6.11 %N	yield : 90%
13		58.73 %C	6.76 %H	6.17 %N	88%
14		58.79 %C	6.65 %H	6.09 %N	89%
15		58.88 %C	6.77 %H	6.04 %N	90%

DCI/NH3- data (relative intensities are given in parenthesis)

11	$[MH^+]$:	212	(100)	$[B + 30]^+$:	108	(4)
	$[B + 44]^+$:	122	(4.2)			
12	$[MH^+]$:	226	(100)	$[B + 30]^+$:	122	(6.6)
	$[B + 44]^+$:	136	(4.2)			
13	$[MH^+]$:	226	(100)	$[B + 30]^+$:	122	(8.7)
	$[B + 44]^+$:	136	(7.6)			
14	$[MH^+]$:	226	(100)	$[B + 30]^+$:	122	(3.6)
	$[B + 44]^+$:	136	(4.6)			
15	$[MH^+]$:	226	(100)	$[B + 30]^+$:	122	(9.4)
	$[B + 44]^+$:	136	(10.6)			

3.3. IN VITRO ANTITUMOR EVALUATION.

Tumor L1210, FM3A, Raji, Molt/4F cells were grown in static suspension cultures in Fischer's medium with 10% heat-inactivated (56°, 30 min) horse serum, without antibiotics. The remaining culture conditions and the method of obtaining growth curves were as described previously[5,8]. Data was collected over a 3-day period, which was approximately 6 population doubling times in control cultures. The log of the cell number was plotted against time, and the growth rate for treated cultures was defined as the slope, as a percentage of the slope for a controle culture.

3.4. INHIBITION OF VIRUS-INDUCED CYTOPATHOGENICITY.

Confluent cell cultures in Falcon microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After 1 hour of virus adsorption,residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, µg/ml) of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus infected cell cultures.

ACKNOWLEDGEMENTS.

This work is also supported by NATO-grant 824/84 ; and was aided by grant number CH-312 from the American Cancer Society. One of us Y. Vrijens thanks the IWONL for financial support. We are indebted to Mrs. M. Keirsmaekers, Mr. Y. Luyten and Mr. J. Schrooten, Mrs. A. Van Lierde, Mrs. F. De Meyer, Mrs. L. Van Berckelaar, Mr. W. Van Dongen for technical assistance.

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Received March 10, 1986.