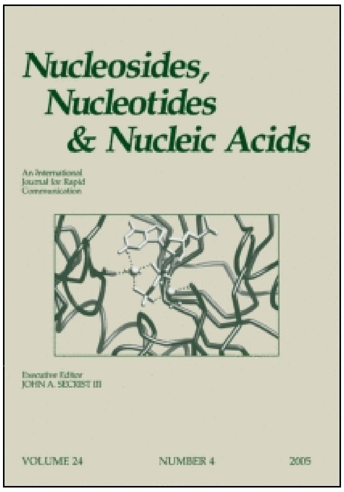


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Novel Isothiazole and Isothiazolo[4,5-*d*] Pyrimidine Analogues of the Natural *C*-Nucleosides Pyrazofurin and the Formycins

Diederik K. Buffel[a]; Lieven Meerpoel[a]; Suzanne M. Toppet[a]; Georges J. Hoornaert[a]
[a] Laboratorium voor Organische synthese, Departement Scheikunde, Katholieke Universiteit Leuven, Heverlee-Leuven, Belgium

To cite this Article Buffel, Diederik K. , Meerpoel, Lieven , Toppet, Suzanne M. and Hoornaert, Georges J.(1994) 'Synthesis of Novel Isothiazole and Isothiazolo[4,5-*d*] Pyrimidine Analogues of the Natural *C*-Nucleosides Pyrazofurin and the Formycins', Nucleosides, Nucleotides and Nucleic Acids, 13: 1, 719 — 736

To link to this Article: DOI: 10.1080/15257779408013275

URL: http://dx.doi.org/10.1080/15257779408013275

NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 719-736 (1994)

SYNTHESIS OF NOVEL ISOTHIAZOLE AND ISOTHIAZOLO[4,5-*d*] PYRIMIDINE ANALOGUES OF THE NATURAL *C*-NUCLEOSIDES PYRAZOFURIN AND THE FORMYCINS

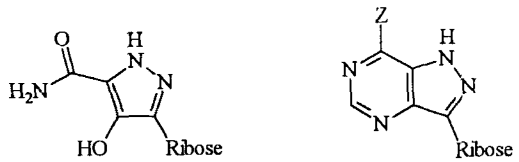
Diederik K. Buffel, Lieven Meerpoel, Suzanne M. Toppet, Georges J. Hoornaert*
Laboratorium voor Organische synthese, Departement Scheikunde , Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Heverlee-Leuven, Belgium

ABSTRACT:

The cycloaddition of tri-*O*-benzyl-2,5-anhydro-D-allononitrile-N-sulfide with dimethyl acetylenedicarboxylate or with dimethyl fumarate followed by DDQ oxidation was found to give the benzoyl protected dimethyl 3-ß-D-ribofuranosyl-isothiazoledicarboxylate **10**. This compound was converted to C-nucleosides **7a**, **8** and **9**, analogues of pyrazofurin, oxoformycin B and formycin respectively. Despite their structural similarities they did show neither antiviral nor antitumor activity.

Interest in nucleoside analogues relates to their possible antiviral and antitumor activity.[1] Several compounds have been prepared by modifying either the sugar moiety or the heterocycle. Particularly of interest in the latter respect are the *C*-nucleosides;[2] due to resistance to hydrolysis of the glycoside linkage these compounds have an enhanced biological stability. An interesting compound in this field is pyrazofurin **1**,[3] a natural *C*-nucleoside isolated from *S. Candidus* and synthesized by several groups.[4] It has a broad spectrum antiviral[5a,b] and antitumor[5c] activity. A number of analogues have been synthesized[6] and some thiophene[7a] and isoxazole[7b] analogues were prepared in our laboratory. The formycins **2a** and **2b**, *C*-nucleoside analogues of adenosine and inosine, interfere extensively with the nucleic acid metabolism.[8] Several synthetic routes for these natural products and their analogues have been developed.[9] Formycin A inhibits the *de novo* purine synthesis[10] and formycin B is a potent inhibitor of purine nucleoside phosphorylase in human erythrocytes.[11] Compound **2a** has antineoplastic activity and inhibits growth of bacteria, fungi and viruses. Formycin B inhibits the growth of mouse sarcoma 180 cell and of influenza A_1 virus. Some extremely toxic analogues have been synthesized.[12]

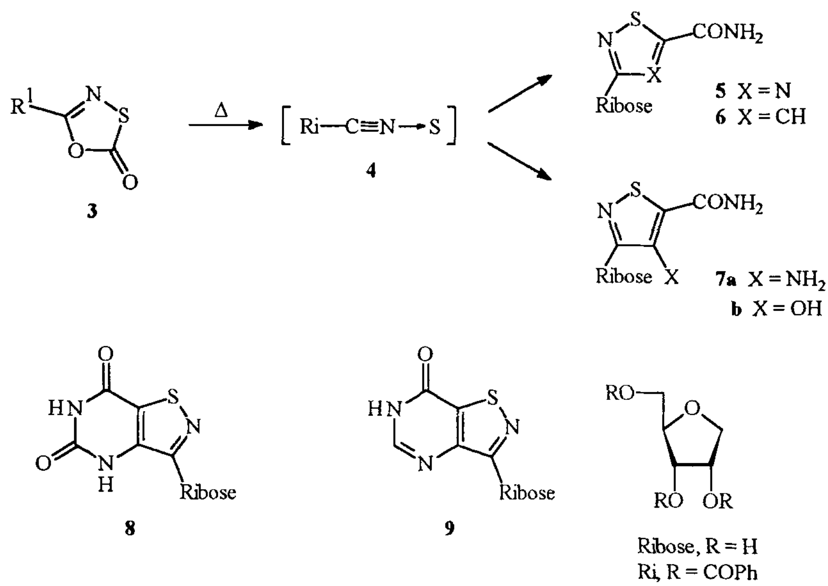
Dedicated to the memory of Professor R.K. Robins.



1 Pyrazofurin

2a Z = NH_2 formycin A
2b Z = OH formycin B

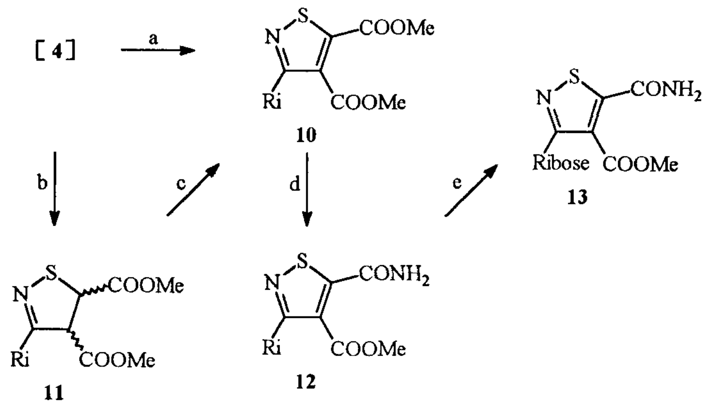
FIG. 1



Scheme 1

Some time ago we studied the decomposition of the oxathiazolone **3** and the use of the D-allononitrile-N-sulphide **4** in the synthesis of a thiadiazole (**5**) and an isothiazole analogue (**6**) of ribavirin.[13] Now we wish to report our work on the synthesis of 4-amino-3-ß-D-ribofuranosyl-5-isothiazole carboxamide **7a**, an analogue of pyrazofurin, and of the isothiazole[4,5-*d*]pyrimidines **8** and **9**, analogues of the formycins (Scheme 1).

Thermolysis of the oxathiazolone **3** with dimethyl acetylenedicarboxylate at 140°C gave rise to low yields of the isothiazole diester **10** (Scheme 2). Side products were the corresponding D-allononitrile, sulphur and benzoic acid (elimination of the

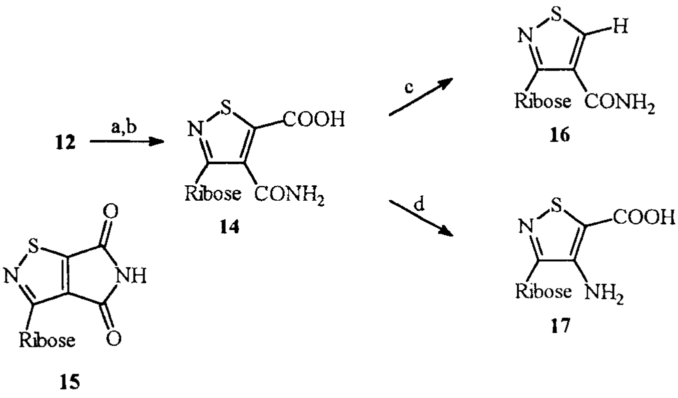


(a) DMAD, 140°C, 6 days (8%); (b) dimethyl fumarate, 200°C, 45 min (58%); (c) DDQ, chlorobenzene, Δ, 4.5h (85%); (d) $CHCl_3/CH_3OH$, 0°C, NH_{3gas}, 16 h (80%); (e) $CHCl_3/CH_3OH$, NaOH (79%).

Scheme 2

latter is a major fragmentation process in the mass spectra of benzoylated nucleosides). Better results were obtained with dimethyl fumarate as the dipolarophile. Upon heating of **3** in neat dimethyl fumarate at 200°C two isomeric isothiazolines **11a,b** were formed in about equal amounts. They were readily separated on a silica gel column with 5% ethyl acetate in toluene; they showed very similar mass, ^{1}H-NMR and ^{13}C-NMR spectra. From the ^{1}H-NMR spectrum it appeared that both compounds had the expected trans configuration at the C_4-C_5 isothiazoline bond : the corresponding coupling constants were 4 Hz as in the analogous trans 3-phenyl-2-isothiazolinecarboxylate;[14] the cis isoxazolines show a $^3J_{4,5}$ of 11.5 Hz.[15] Oxidation of either compound with DDQ (2,3-dicyano-5,6-dichloro-1,4-benzoquinone) gave the isothiazole dicarboxylate **10**. These data clearly indicate that both compounds were the 4R,5S- and 4S,5R-diastereomers; so, in further reactions the crude reaction mixture obtained after heating **3** in dimethyl fumarate was only subjected to flash chromatography to remove tarry material. After oxidation with DDQ in refluxing chlorobenzene and chromatography compound **10** was obtained in 60% overall yield starting from the oxathiazolone **3**.

Careful ammonolysis (monitored on TLC) of this diester **10** with a saturated solution of anhydrous ammonia in a 1:1 mixture of chloroform and methanol cooled in ice water, gave the monoamide **12** : of both ester functions only one, most probably the isothiazole-5-position, had reacted. This position is presumably more activated (by the



(a) CH_3OH/CH_3ONa, rt., 1.5 h (89%); (b) Dowex 50W-X2, H^+; (c) 130°C; (d) (1) $Ba(OH)_2$, Br_2, 5-10°C, (2) H_2SO_4 (95%)

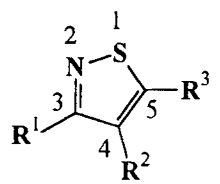
Scheme 3

conjugated ring nitrogen) than the 4-position (see for example the analogous regioselective nucleophilic reactions on isothiazole[16] and pyrazole dicarboxylate esters [9a]). Debenzoylation of **12** to free nucleoside **13** could be effected with a catalytic amount of sodium hydroxide in methanol-chloroform. Treatment of **12** with excess sodium methoxide followed by work-up with Dowex 50W-X2(H^+) yielded a new compound which was assigned the 4-carbamoyl-3-ß-D-ribofuranosyl-5-isothiazolecarboxylic acid structure **14** (Scheme 3). After decarboxylation by heating, a new compound was isolated and shown to be identical (TLC, spectra) with the previously obtained 3-ß-D-ribofuranosyl-4-isothiazolecarboxamide **16**.[13]

We believe that compound **14** is formed via a cyclic imide **15** as it is known that phtalic imide can be hydrolysed to phtalic acid amide by acid and by base.[17] Ring opening of **15** should again occur by nucleophilic attack at the more electrophilic 5-position to give compound **14**. The ease of decarboxylation of **14** is also in agreement with Adams and Slack's observations that isothiazole-4,5-dicarboxylic acid is decarboxylated at the 5-position. Removal of the second carboxyl group occurred much less readily.[16]

Hofmann rearrangement of the 4-carbamoyl function in **14** to the amine **17** was effected with barium hypobromite[18] at 60°C. After neutralization with sulphuric acid, filtering of the barium sulphate and purification on Dowex 50W-X2(H^+), the pure

TABLE 1. ^{13}C-NMR chemical shifts (ppm) for substituted isothiazole derivatives



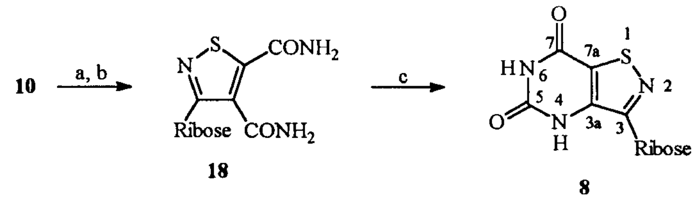
R^1	R^2	R^3	C_3	C_4	C_5
H	H	H[a]	157	123	150
Ribose	NH_2	COOH(17)	159	147	126
Ribose	NH_2	$CONH_2$(7a)	159	147	128
Methyl	COOMe	NH_2[b]	168	106	180

[a] from ref. 19.

[b] material kindly provided by Prof. Dr. J. Goerdeler

compound **17** was isolated as an oil in excellent yield. The ^{13}C-NMR spectrum is in agreement with the proposed structure. The isothiazole-C absorptions are presented in Table 1, together with data for some related compounds. The C_4 atom is deshielded with respect to the parent isothiazole,[19] whereas C_5 is shielded as expected from the substituent effects on the carbon absorptions in benzene[20] : the amino group induces an α-shift of +18 ppm and an ortho shift of -13 ppm whereas the carbomethoxy group gives an α-shift of +1.8 ppm and an ortho shift of +1 ppm. The spectrum recorded for methyl 5-amino-3-methyl-4-isothiazole-carboxylate (kindly provided by Prof.dr.J. Goerdeler) shows analogous effects of the 5-amino group clearly distinguishing between the 4- and the 5-amino-isothiazoles. (Table 1)

The diester **10** was also subjected to more vigorous ammonolysis conditions (overnight at room temperature). The resulting complex mixture of partially deblocked material was treated with excess sodium methoxide to remove the benzoyl protection completely. The mixture containing the diamide **18** (Scheme 4) and 20% of the monoamide **14**, was subjected to Hofmann conditions (barium hypobromite) without

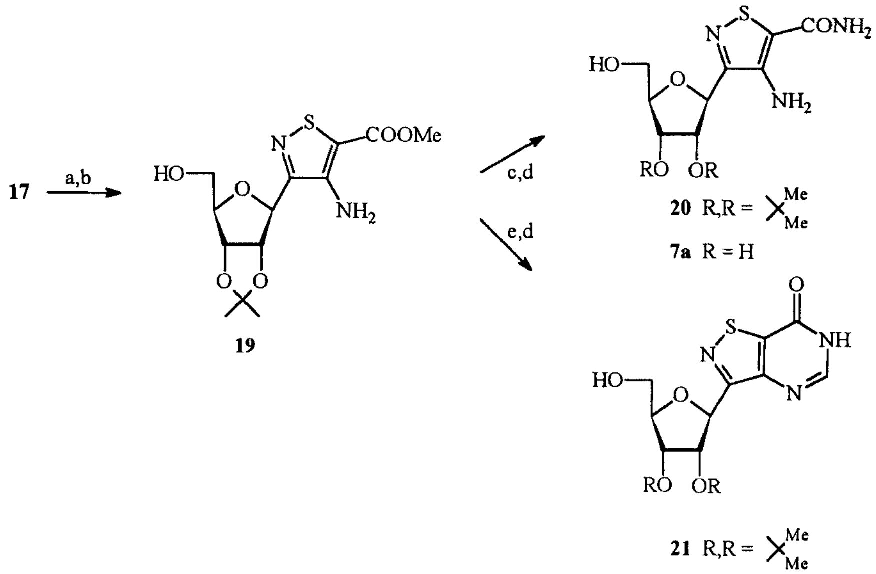


(a) $CHCl_3/CH_3OH$, NH_{3gas}, overnight, rt.; (b) $CH_3OH/NaOCH_3$, rt., 1.5h (73%); (c) (1) $Ba(OH)_2/Br_2$, 60°C, 1h, (2) H_2SO_4 (74%).

Scheme 4

prior purification. After work up the 3-ß-D-ribofuranosyl-isothiazole[4,5-d]pyrimidine-5-(4H)-7(6H)-dione **8**, an analogue of oxoformycin B, was crystallized from water in 74% yield. The structure of this compound could be proved by ^{13}C-NMR data : in DMSO-d_6 five quaternary carbon signals appear, C_3 at 158.2, C_{3a} at 140.1, C_5 at 151.4, C_7 at 157.4 and C_{7a} at 132.9 ppm. The proton coupled spectrum shows long range coupling between the sugar protons, C_3 and C_{3a}. The spectrum of a partially H/D exchanged sample shows apparent doublets at 140.1 and 157.4 ppm and an apparent triplet at 151.4 ppm, caused by an isotope effect from NH/ND on the neighbouring carbon atoms. This type of isotope effects on amides and peptides has been used by Feeney et al. to assign the amide carbon absorptions in oligopeptides.[21] Thus the C_{3a} atom shows both a long range C-H coupling with the ribose protons and an isotope effect from the neighbouring NH group. The long range coupling and the isotope splitting excludes the isomeric [5,4-*d*] structure. This regioselectivity can be explained by the orientation effect of the isothiazole nucleus favouring the Hofmann rearrangement of the more electron rich amide function at position 4.[22] The same type of selectivity was reported for 1-methyl-1,2,3-triazole-dicarboxamide and 3,4-pyrimidinedicarboxamide,[22] although 3,4-isothiazoledicarboxamide did not react in a Hofmann rearrangement[23].

In order to get the 3-ß-D-ribofuranosyl-4-amino-5-isothiazolecarboxamide 7a, compound **17** was treated with methanol and hydrogen chloride followed by reaction with dimethoxypropane and acid to yield the ester **19**. This compound was then reacted with liquid ammonia in a sealed tube at 75°C overnight, giving the protected amide **20** in 22% overall yield starting from compound **17**. Due to the 4-amino function, ammonolysis of the 5-ester group in **19** occurred much less readily than with the dicarboxylate **10**. The ^1H-NMR spectra of the ester **19** and the amide **20** showed a δ



(a) CH_3OH/HCl, 24 h, rt.; (b) $CH_3C(OCH_3)_2CH_3$, HCl/CH_3OH, 4 h (56%); (c) NH_{3liq}, 75°C, 8 h (39%); (d) diox./H_2O, Dowex 50W-X2 H^+, 6 h, (87%); (e) ethyl formimidate, $CH_3OH/NaOCH_3$, 150°C, 2.5 h (61%).

Scheme 5

value for the isopropylidene methyl groups of 0.22 and 0.20 respectively[24] and a multiplet absorption for $H_{4'}$.[25] This is consistent with a ß-configuration at $C_{1'}$. Finally the free nucleoside **7a** was obtained as an oil (87%) after deprotection with Dowex 50W-X2(H^+).

Several attempts to get compounds of type **7b** from compounds **17** and **19**, the deprotected ester or its tri-*O*-(*tert.*-butyl dimethylsilyl)derivative, were not successful. By treatment with sodium nitrite at pH 4, in acetic acid or in hydrochloric acid[26a] (with or without photolysis), or with nitrosyl tetrafluoroborate and acid,[26b] either unprotected material, starting material or complex mixtures were found.

Of course the nucleoside **7a** could be obtained from **17** without using the isopropylidene protection. We preferred however to use the protection in order to facilitate purification and to prove the ß-configuration. Moreover, the protected ester **19** was further used in the synthesis of the formycin B analogue **9** : sodium methoxide catalysed cyclisation of **19** with ethyl formimidate[27,28] in a sealed tube at 150°C gave the protected 3-ß-D-ribofuranosyl-isothiazole[4,5-d]pyrimidine-(6H)-one **21** in 61% yield. Its ^1H-NMR spectrum showed a new singlet absorption at 8.1 ppm for H_5 (the

TABLE 2. Calculated [a] conformational parameters for the ribofuranose moiety of Pyrazofurin **1**, Formycin **2a** and the analogues **7a** and **9**.

compd.	P_N	P_S	$^N\tau_m$	$^S\tau_m$	X_N
1[b]	41.1	141.9	42.0	42.4	0.32
7a	40.0	143.3	41.4	43.0	0.40
2a[c]	29.0	154.0	37.0	35.5	0.27
9	30.0	153.0	39.7	36.7	0.39

[a] from ^1H NMR coupling constants, based on the graphs of ref.30.
The parameters P_N and P_S describe the phase angles of the N and S conformer in the pseudorotational model of ref.31. $^N\tau_m$ and $^S\tau_m$ are the ring pucker values and X_N is the molar fraction of N in the N,S-equilibrium.
[b] based on the spectrum reported in ref.4c.
[c] based on the spectrum reported in ref.32.

corresponding proton in quinazoline absorbs at 8.2 ppm.[29] Again the δ value for the isopropylidene methyls and the multiplicity for the $H_{4'}$ absorption confirmed the ß-configuration. Deprotection with Dowex 50W-X2(H^+) gave white crystals (89%) of the free nucleoside **9**. The ^{13}C-NMR showed five aromatic carbon atoms which could be assigned using the proton decoupled spectrum : C_3 has a multiplet absorption due to long range coupling with the ribose protons and C_{3a} shows a doublet of doublets (coupling with $H_{1'}$ and H_5); C_5 obviously is a doublet, C_7 has a long range coupling with H_5 and C_{7a} is a singlet.

The conformational equilibrium for the ribofuranosyl moiety in the nucleoside analogues **7a** and **9** was calculated from their 250 MHz ^1H-NMR spectra in D_2O solution at room temperature. The first order constants $^JH_{1'2'}$, $^JH_{2'3'}$ and $^JH_{3'4'}$ were subjected to the graphical analysis developed by Davies and Danyluk[30] based on the pseudorotational model of Altona and Sundaralingam.[31] The results are collected in Table 2. For comparison we have included the figures for pyrazofurin and formycin, calculated from the literature spectra.[4c,32] From this table it is clear that the compound **7a** is very similar to pyrazofurin as for phase angle and ring puckering of the ribose moiety. The N/S equilibrium shows a small shift towards the north conformer. The comparable conclusions apply to the isothiazolopyrimidine **9** with respect to formycin **2a**.

TABLE 3. Calculated [a] rotameric distribution at the $C_{4'}$-$C_{5'}$ bond in Pyrazofurin **1**, Formycin **2a** and the analogues **7a** and **9**.

compd.	%gg	%gt	%tg
1[b]	60	40	00
7a	62	32	06
2a[c]	67	25	08
9	62	30	08

[a] from ^{1}H NMR coupling constants, according to Haasnoot's method.

%gg, %gt, %tg are the gauche-gauche, gauche-trans and trans-gauche ($H_{4'}H_{5'}$,$H_{4'}H_{5''}$) conformer populations respectively.

[b] based on the reported spectrum in ref.4c.

[c] based on the reported spectrum in ref.32.

We have also calculated the rotameric distribution at the $C_{4'}C_{5'}$-exocyclic bond using Haasnoot's method.[33] The AB part of the $H_{4'}$ $H_{5'}$ $H_{5''}$ ABX spectrum was subjected to second order analysis[34] to obtain the accurate coupling constants. The rotameric populations are presented in table 3 together with the parameters for pyrazofurin and formycin obtained from the literature spectra **5c**.[32] The similarity between the natural nucleosides and the analogues is again striking.

The biological activities of compounds **7a**, **8**, **9**, **13** and **17** were tested. Notwithstanding the conformational similarities to pyrazofurin and formycin, no significant inhibition of either in vitro L1210 cell growth or of viral replication (HSV-1, measles, polio-1, VSV, vaccinia, reovirus-1, parainfluenzavirus-3 or cocksackievirus-B4) could be detected.

EXPERIMENTAL

Materials and Methods

Melting points, determined with a Leitz melting point microscope are uncorrected. The ^{1}H-NMR data are presented in ppm downfield from Me_4Si used as an internal standard; the spectra were taken on a Varian EM 390 spectrometer at 90MHz and a Bruker WM 250 at 250 MHz; for the ^{13}C-NMR spectra a Bruker WM 250 and a

Bruker WP 80 spectrometer were used. The mass spectra were recorded on a Kratos-MS-907-S apparatus with direct insertion and an ionization energy of 70 eV. Infrared spectra were obtained from a Perkin-Elmer 250 grating apparatus. For the chromatographic separations Merck silicagel 60 (0.063-0.200 mm) was used unless otherwise stated.

4-amino-3-ß-D-ribofuranosyl-5-isothiazolecarboxamide **7a**. A solution of **20** (79.5 mg, 0.25 mmol) in dioxane (2 ml) was stirred with dowex 50W-X2 (H^+) for 6 h at room temperature. After filtration and evaporation the residue was lyophilized to give 62 mg of **7a** as a slightly coloured powder (87%). IR (KBr), 3500 cm^{-1}; ^{1}H-NMR (D_2O) δ 4.93 (d, J=6.4 Hz, $H_{1'}$), 4.45 (dxd, J=6.4 Hz, J=5.5 Hz, $H_{2'}$), 4.22 (dxd, J=5.5 Hz, J=4.6 Hz, $H_{3'}$), 4.10 (m, $H_{4'}$,) 3.83 (dxd, J=3 Hz,J=-12.1 Hz, $H_{5'}$) 3.75 (dxd, J=4.4 Hz, J=-12.1 Hz, $H_{5''}$); ^{13}C-NMR (D_2O) δ 166.4 ($CONH_2$), 159.1 (C_3), 146.8 (C_4), 127.8 (C_5), 86.1, 81.5, 74.4, 72.1, 62.5 (ribose C); MS (+5 Me_3Si), m/z 635, 620, 545, 502, 440, 230, 217, 103, 72.

3-ß-D-ribofuranosyl-isothiazolo[4,5-*d*]pyrimidine-5(4*H*)-7(6*H*)-dione **8**. A mixture (501 mg) of diamide **18** and acid **14** was dissolved in a barium hypobromite solution (prepared as mentioned for the synthesis of **17**) and was heated at 60°C for 1 h. After cooling the solution was acidified with sulphuric acid (3 N) and filtered over celite. The filter was washed with water (100 ml) and filtrate and washings were brought to pH 9 with K_2CO_3. After evaporation the mixture was dissolved in water (10 ml) and purified on a dowex 50W-X2 column with water, giving 295 mg of **8** (74%) as white crystals; mp 225-227°C; ^{1}H-NMR (dmso-d_6) δ 11.6 (s_{br}, NH), 11.4 (s_{br}, NH), 4.94 (d, J=8 Hz, $H_{1'}$), 4.14 (dxd, J=8 Hz, J=6 Hz, $H_{2'}$), 4.05 (m, $H_{3'}$, $H_{4'}$), 3.67 (d, J=1 Hz, $H_{5'}$, $H_{5''}$); ^{13}C-NMR (dmso-d6) δ 158.2 (C_3), 157.4 (C_7), 151.4 (C_5), 140.1 (C_{3a}), 132.9 (C_{7a}), 86.1, 82.0, 81.6, 74.6, 72.2, 61.4 (ribose C); MS (+ 3 Me_3Si) m/z 517, 502, 474, 446, 427, 414, 356, 337, 296, 268, 217, 103, 73.

3-ß-D-ribofuranosyl-isothiazolo[4,5-*d*]pyrimidine-7(6*H*)-one **9**. The isopropylidene derivative **21** (46.1 mg, 0.14 mmol) was dissolved in water (2 ml) and stirred with Dowex 50W-X2 (H+) for 6.5 h at room temperature. After filtration, washing and evaporation of the filtrates and washings, compound **9** was obtained. Recrystallization from water gave 36 mg (89%) of white crystals; mp 132-134°C; ^{1}H-NMR (D_2O) δ 8.29 (s, H_5), 5.33 (d, J=6.0 Hz, $H_{1'}$), 4.61 (dxd, J=6.0 Hz, J=5.8 Hz, $H_{2'}$), 4.46 (dxd, J=5.8 Hz, J=3.9 Hz, $H_{3'}$), 4.35 (m, $H_{4'}$), 3.92 (dxd, J=3.1 Hz, J=-12.8 Hz, $H_{5'}$), 3.83 (dxd, J=4.3 Hz, J=-12.8 Hz, $H_{5''}$); ^{13}C-NMR (D_2O, assigned using the 1H coupled spectrum) δ 164.3 (C_3), 158.8 (C_7), 151.0 (C_{3a}), 148.5 (C_5), 142.9 (C_{7a}), 86.2, 81.0, 75.6, 72.6, 62.9, (ribose C); MS (+ 3 Me_3Si), m/z 501, 486, 411, 398, 340, 280, 259, 258, 254, 252, 230, 217, 182, 103, 73; Anal. Calcd for $C_{10}H_{11}O_5N_3S$. ½ H_2O: C, 40.85; H, 3.97; N, 14.19. Found: C, 40.81; H, 4.09; N, 14.28.

Dimethyl 3-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)-4,5-isothiazoledicarboxylate **10**.

Method A (with dimethyl acetylenedicarboxylate). Oxathiazolone **3**[13] (0.547 g, 1 mmol) was suspended in dimethyl acetylenedicarboxylate (1 ml, 8.1 mmol) and heated at 140°C under argon for 6 days. Excess dipolarophile was removed under reduced pressure and the residue was separated on a preparative silica gel plate (eluent : 10% ethyl acetate in benzene) to give 51 mg (8%) of the isothiazole diester **10** as a slightly yellow oil. This compound was identical (^{1}H-NMR, mass spectrum and TLC characteristics) with the material obtained in method B.

Method B (with dimethyl fumarate and oxidation with DDQ). Oxathiazolone **3** (10.0 g, 18.2 mmol) was added portionwise to a stirred melt of dimethyl fumarate (40 g, twice recrystallized from cyclohexane) heated in an oil bath at 130°C. After complete dissolution the bath temperature was raised to 200°C; the mixture was then refluxed for 1 h and cooled. After removal of excess fumarate under reduced pressure (15 torr, 100°C), the residue was purified by flash chromatography on a silica gel column with 12% ethyl acetate in toluene. The solvent was then evaporated and the residue was dissolved in chlorobenzene (100 ml). After addition of DDQ (4.0 g, 17.6 mmol), the solution was refluxed for 3.5 h (bath temperature 140°C). The solvent was then removed under vacuum and the residue was subjected to flash chromatography over a silica gel column with 20% ethyl acetate in toluene. The solution was concentrated under reduced pressure and chromatographed on a silica gel column (0.040-0.063 mm with 1 tot 10% ethyl acetate in toluene). The fractions containing compound **10** were pooled and evaporated to give 7.28 g (62%) of the diester as a hygroscopic non crystallizable oil: ^{1}H-NMR ($CDCl_3$) δ 8.1-7.8 (m, ArH), 7.6-7.2 (m, ArH), 6.32 (dxd, J=3.4 Hz, J=4.9 Hz, $H_{2'}$), 5.92 (t, J=4.9 Hz, $H_{3'}$), 5.65 (d, J=3.4 Hz, $H_{1'}$), 4.9-4.4 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 3.90 (s, CH_3OOC); MS m/z 645, 614, 523, 445, 401, 201, 105 : exact mass calcd. for $C_{33}H_{27}NO_{11}S$: 645.130, found 645.131 ± 0.001.

Dimethyl 4R, 5S and 4S, 5R-3-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)-isothiazoline-4,5-dicarboxylates **11a,b**. Oxathiazolone **3** (5.58 g, 10.2 mmol) and dimethyl fumarate (21.5 g twice recrystallized from cyclohexane) were reacted as above. After work-up the residue was chromatographed on a silica gel column (0.040-0.063 mm) with 5% ethyl acetate in toluene. After evaporation of the homogeneous fractions 1.41 g (21.5%) of isomer A and 2.41 g (37%) of isomer B were obtained as oils. Isomer A : ^{1}H-NMR ($CDCl_3$) δ 8.15-7.82 (m, ArH), 7.6-7.1 (m, ArH), 6.05 (dxd, J=3.5 Hz; J=5.5 Hz, $H_{2'}$), 5.76 (t, J=5.5 Hz, $H_{3'}$), 5.20 (d, J=3.5 Hz, $H_{1'}$) 4.86 and 4.79 (2xd, J=5.2 Hz), 4.7 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 3.7 (s, CH_3OOC); ^{13}C-NMR ($CDCl_3$, assigned using the ^1H-coupled spectrum and via decoupling at 3.7 ppm in the proton area) δ 170.2, 167.8 ($COOCH_3$), 166.0 (C_3), 165.2, 165.0, 162.0 (COOPh), 133.3-128.2 (ArC), 81.0 (C_1), 79.3 ($C_{4'}$), 74.0 ($C_{2'}$), 71.9 ($C_{3'}$), 63.6 ($C_{5'}$), 60.4 and 52.6 (C_4 and C_5), 53.0 (q, CH_3OOC); MS m/z 647, 645, 616, 614, 588, 525, 523, 445, 403, 401, 105. Isomer B : ^1H-NMR ($CDCl_3$) δ 8.2-7.8 (m, ArH), 7.5-7.2 (m, ArH), 6.0 (dxd, J=4 Hz; J=5 Hz, $H_{2'}$), 5.83 (t, J=5 Hz), $H_{3'}$), 5.27 (d, J=4 Hz, $H_{1'}$), 5.03 (d, J=4.5 Hz, H_4 or H_5), 4.74 (d, J=4.5 Hz, H_5 or H_4), 4.9-4.5 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 3.73 (s, CH_3OOC), 3.69 (s, CH_3OOC); ^{13}C-NMR ($CDCl_3$, assigned via the proton coupled spectrum and via decoupling at 3.7 ppm in the proton area) 170.2, 168.2 (2xs, $COOCH_3$), 166.2 (s, C_3), 165.3, 165.3, 162.8 (s, COOPh), 133.5-128.5 (ArC), 80.5 ($C_{1'}$), 80.0 ($C_{4'}$), 74.2 ($C_{2'}$), 72.7 ($C_{3'}$), 64.2 ($C_{5'}$), 59.5, 52.4 (C_4, C_5), 53.2 (CH_3OOC); MS m/z 647, 645, 616, 614, 588, 525, 523, 445, 403, 401, 105.

When either compound was oxidized with DDQ the isothiazole **10** was obtained in 86% and 85% yield.

Methyl-5-carbamoyl-3-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)-isothiazole-4-carboxylate **12**. The diester **10** (13.36 g, 20.7 mmol) was dissolved in a mixture of $CHCl_3$ (150 ml) and CH_3OH (150 ml), cooled in an ice bath and saturated with ammonia. After 2 h at 0°C the solvent was removed under reduced pressure and the residue was chromatographed over silica gel with 10 to 50% ethyl acetate in toluene. The fractions containing the amide **12** were evaporated, leaving 10.53 g of an amorphous compound (80.2%) : ^1H-NMR ($CDCl_3$) δ 8.49 (s_{br}, NH), 8.1-7.8 (m, ArH), 7.6-7.2 (m, ArH), 6.54 (s_{br}, NH), 6.33 (dxd, J=2.5 Hz, J=5 Hz, $H_{2'}$), 5.92 (dxd, J=5 Hz, J=7.0 Hz, $H_{3'}$), 5.76 (d,J=2.5 Hz, $H_{1'}$), 4.7 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 3.93 (s, CH_3OOC); MS m/z 630, 599, 508, 495, 445, 105. Anal. Calcd. for $C_{32}H_{26}N_2O_{10}S$: C, 60.95; H, 4.16; N, 4.44; S, 5.08. Found : C, 61.30; H, 4.14, N, 4.33; S, 5.08.

Methyl-5-carbamoyl-3-ß-D-ribofuranosyl-isothiazole-4-carboxylate **13**. The protected monoamide **12** (0.15 g; 0.24 mmol) was dissolved in $CHCl_3$ (2 ml) and NaOH (0.15 mmol) in CH_3OH (2 ml) was added. The mixture was kept at room temperature for 20 min and was then neutralized with Dowex 50 W-X2 (H^+), filtered and evaporated under reduced pressure. The residue was dissolved in water and extracted with $CHCl_3$, the water layer was concentrated to give 60 mg (79%) of a clear sirup ; IR (KBr) 3300 (OH, NH_2), 1710 ($COOCH_3$), 1660, 1590 cm^{-1} ($CONH_2$); 1H-NMR (D_2O) δ 5.42 (d, J=3.5 Hz, $H_{1'}$), 4.41 (dxd, J=3.5 Hz, J=4.2 Hz, $H_{2'}$), 4.22 (t, J=4.2 Hz, $H_{3'}$), 4.17 (m, $H_{4'}$), 3.95 (s, CH_3OOC), 3.8 (m, $H_{5'}$, $H_{5''}$); ^{13}C-NMR (D_2O), 169.1, 167.2, 164.7, 164.5 ($CONH_2$, $COOCH_3$, C_3, C_5), 127.8 (C_4) 84.7 ($C_{1'}$), 82.4 ($C_{4'}$), 76.2, 71.6 ($C_{2'}$, $C_{3'}$), 62.7 ($C_{5'}$), 54.2 (CH_3OOC); MS (+4 Me_3Si), m/z 606, 591, 503, 217, 215, 73.

4-carbamoyl-3-ß-D-ribofuranosyl-isothiazole-5-carboxylic acid **14.** The protected monoamide **12** (10.48 g, 16.6 mmol) was treated with 200 ml absolute methanol containing sodium methoxide (from 2.21 g Na, 96 mmol) at room temperature. The reaction was monitored by TLC (cellulose, 20% 0.2 M NH_4OAc - 80% Me_2CO) and was complete after 75 min. The mixture was treated with Dowex 50 W-X2 (H^+)-which gave an acid solution - and concentrated under vacuum.

The residue was dissolved in water, extracted with $CHCl_3$ (3 x 50 ml) decolorized with active carbon, filtered over celite and evaporated to give the acid **14** as an oil (4.50 g, 89%). The compound was crystallized from water, mp 103-107°C (dec at 112°C); IR (KBr) 3300 (OH, NH_2), 1710 (COOH), 1670, 1615 cm^{-1} ($CONH_2$); ^1H-NMR (D_20), δ 5.15 (d, J=6 Hz, $H_{1'}$), 4.45 (t, J=6 Hz, $H_{2'}$), 4.25 (t, J=6 Hz, $H_{3'}$), 4.1 (m, $H_{4'}$), 3.8 (m, $H_{5'}$, $H_{5''}$); MS (+5 Me_3Si) m/z 664, 649, 574, 533, 484, 471, 299, 217, 73.

When this compound was heated at 130°C, a product identical (TLC, ^1H-NMR and mass spectrum) with 3-ß-D-ribofuranosylisothiazole-4-carboxamide **16**[13] was obtained.

4-Amino-3-ß-ribofuranosyl-isothiazole-5-carboxylic acid **17**. $Ba(OH)_2.8H_20$ (4.36 g, 13.8 mmol) was dissolved in demineralized water (100 ml) at 60°C; insoluble barium carbonate was removed by filtration. To the cooled (5-10°C) filtrate bromine (0.53 g, 3.3 mmol) was added. The mixture was stirred until all bromine was dissolved and the amide **14** (760 mg, 2.5 mmol) was then added. After 45 min at 60°C all hypobromite had disappeared (KI-H_2SO_4 test). The amide was converted to a single new compound (TLC on cellulose, 30% 0.2 M NH_4OAc-$(CH_3)_2CO$). The cooled solution was neutralized with 3 N sulphuric acid and the precipitate was removed by cellite filtration. The filtrate was concentrated under reduced pressure to 25 ml and passed through a Dowex column 50W-X4 (200-400 mesh). First a strongly acidic fraction was washed off with water and the amine was eluted with 1 N ammonia. The fractions containing the amine **17** were evaporated and lyophilized to give 655 mg (95%) of an oily compound : IR (KBr) 3300, 1690, 1610 cm^{-1}; ^1H-NMR (D_20) δ 4.95 (d, J=6 Hz, H_1), 4.42 (t, J=4 Hz, $H_{2'}$), 4.15 (m, $H_{3'}$, $H_{4'}$), 3.8 (m, $H_{5'}$, $H_{5''}$); ^{13}C-NMR (D_20) δ 165.3 (COOH), 158.9 (C_3), 147.0 (C_4), 125.7 (C_5), 85.9, 81.9, 74.5, 72.1, 62.4 (ribose C); MS (+ 4 Me_3Si) m/z 564, 549, 474, 432, 384, 371, 331, 230, 217, 73.

3-ß-D-ribofuranosyl-isothiazole-4,5-dicarboxamide **18**. A solution of diester **10** (4.53 g, 7 mmol) in a mixture of methanol (50 ml) and chloroform (50 ml) was saturated with NH_3 gas at 0°C, and kept overnight at room temperature. The solvent was evaporated and the residue was treated with $NaOCH_3$ in methanol (from 1.00 g

NaH in 30 ml methanol). After 90 min at room temperature the mixture was neutralized with Dowex 50W-X2 (H+) and evaporated. The residue was dissolved in water (50 ml) and extracted with chloroform (3x50 ml). The water layer was lyophilized to give 2.01 g of a 4 to 1 mixture (according to ^{1}H-NMR) of the diamide **18** and the acid **14**; ^{1}H-NMR (D_2O) δ 5.10 (d, 5.4 Hz, $H_{1'}$), 4.50 (t, 5.4 Hz, $H_{2'}$), 4.22 (dxd, J=5.4 Hz, J=5.1 Hz, $H_{3'}$), 4.07 (m, $H_{4'}$), 3.8 (m, $H_{5'}$, $H_{5''}$); chemical ionisation MS (+5 Me_3Si), m/z 664 (MH^+).

Methyl 4-amino-3-(2', 3'-*O*-isopropylidene)-ß-D-ribofuranosyl-isothiazole-5-carboxylate **19**. Aminoacid **17** (127 mg, 0.646 mmol) was treated with a saturated solution of HCl gas in dry methanol at room temperature for several days. Then the solvent was removed in vacuo and the residue was dissolved in dimethoxypropane - HCl (0.8 M, 4 ml) and 1 ml of dry methanol was added. After 4 h the mixture was neutralised with 5% aqueous $NaHCO_3$ and extracted with chloroform. The organic layer was dried on $MgSO_4$ and evaporated and the residue was purified on a preparative silica gel plate to give 85 mg (56%) of the ester **19** as a colourless oil; ^{1}H-NMR ($CDCl_3$) δ 5.85 (s_{br}, NH_2), 5.0 (m, $H_{1'}$, $H_{2'}$), 4.76 (dxd, J=3 Hz, J=6 Hz, $H_{3'}$), 4.22 (m, $H_{4'}$), 3.87 (s, CH_3OOC), 3.75 (m, $H_{5'}$,$H_{5''}$, OH), 1.60 (s, CH_3), 1.38 (s, CH_3); MS m/z 330, 315, 300, 299, 272, 242, 187, 59, 42.

4-Amino-3-(2',3'-O-isopropylidene)-ß-D-ribofuranosyl-isothiazole-5-carboxamide **20**. Compound **19** (106 mg, 0.32 mmol) was dissolved in liquid ammonia in a sealed tube and heated in a bomb for 8 h at 75°C. The ammonia was slowly evaporated and the residue was separated on a preparative silica gel plate (with 10% methanol in chloroform) to give 40 mg of the amide **20** (39%). Another fraction contained 29.6 mg (28%) of the starting material; IR (oil film) 3500-3150 (OH, NH_2CO, NH_2), 1660, 1600 cm^{-1} ($CONH_2$); ^{1}H NMR ($CDCl_3$) δ 5.93, 5.83 (2 x s_{br}, NH_2 and $CONH_2$), 5.1 (m, $H_{1'}$, $H_{2'}$), 4.80 (dxd, J=2.5 Hz, J=4.5 Hz, $H_{3'}$), 4.30 (m, $H_{4'}$,), 3.9 - 3.4 (m, $H_{5'}$, $H_{5''}$, OH), 1.60 (s, CH_3), 1.40 (s, CH_3); mass spectrum, m/z 315, 300, 297, 285, 257, 59, 43.

3-(2',3'-O-isopropylidene)-ß-D-ribofuranosyl-isothiazolo[4,5-*d*]-pyrimidine-7(6*H*)-one **21**. A mixture of compound **19** (88 mg, 0.26 mmol), ethyl formimidate (4 ml) and sodium methoxide in methanol (from 0.8 mg NaH in 0.2 ml methanol) was heated at 150°C in a sealed tube for 2.5 h. The reaction mixture was evaporated and purified on a preparative silica gel plate (5% methanol in chloroform) to give 52 mg of **21** as an oil (61%): ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.1 (s, H_5), 5.46 (d, J=6 Hz, $H_{1'}$), 5.04 (m, $H_{2'}$, $H_{3'}$), 4.47 (m, $H_{4'}$), 4.3 (s_{br}, OH and NH), 3.85 (m, $H_{5'}$, $H_{5''}$), 1.70 (s, CH_3) 1.41 (s, CH_3); MS m/z 325, 310, 307, 292, 267, 237, 182, 151.

Acknowledgement.

The authors are indebted to the "Fonds voor Kollektief Fundamenteel Onderzoek", the "Ministerie voor Wetenschapsbeleid" and the "Onderzoeksfonds KU Leuven" for financial support. They thank Prof. E. De Clercq (Rega Institute, K.U.Leuven) for the biological testing, Dr. F. Compernolle and R. De Boer for mass spectral analyses and P. Valvekens, Ph. Van Parijs and D. Stevens for technical assistance. The N.F.W.O. and I.W.O.N.L. are acknowledged for the research grants (D.B. and L.M.).

REFERENCES

1) a) Dolin, R., *Science* **1989**, 227, 1296. b) De Clercq, E.D.A., *I.S.I. Atlas Sci. Pharmacol.* **1987**, 1, 20. c) Montgomery, J.A., *Antiviral Res.* **1989**, 12, 113. d) De Clercq, E., *Antiviral Res.* **1989**, 12, 1. e) Mitsuya, H., Yarchoan, R., Broder, S., *Science* **1990**, 249, 1533. f) De Clercq, E., *Nucleosides and Nucleotides* **1991**, 10, 167.

2) For a review on C-nucleosides see: a) Hanessian, A., Pernet, A.G., *Adv. Carbohydr. Chem. (Biochem.)*, **1976**, 33 111; b) James, S.R., *J. Carbohydr., Nucleosides, Nucleotides* **1979**, 6, 417. c) Knutsen, L.J.S., *Nucleosides and Nucleotides* **1992**, 11, 961.

3) For a description of the discovery, synthesis and (bio)chemical properties of pyrazofurin, see: a) Suhadolnik, R.J., *"Nucleoside antibiotics"*, Wiley, New York, **1970**, 390; b) , Suhadolnik, R.J. *"Nucleosides as biological probes"*, New York, **1979**, 281; and references cited therein.

4) a) Farkas, J., Flegelova, A., Sorm, F., *Tetrahedron Lett.* **1972**, 2279; b) De Bernardo, S., Weigele, M., *J. Org. Chem.* **1976**, 41, 287; c) Buchanan, J.G., Stobie, A., Wightman, R.H., *J. Chem. Soc. Perkin Trans. I*, **1981**, 2374; d) Katagiri, N., Takashima, K., Kato, T., *Nucl. Acid. Res. Symp. Ser.* **1982**, 11, 137.

5) a) Gutowski, G.E., Sweeney, M.J., De Long, D.C., Hamill, R.L., Gerzon, K., Dyke, R.W., Ann. N.Y. *Acad. Sci.* **1975**, 255, 544; b) Descamps, J., De Clercq, E., *Current Chemotherapy* **1978**, 354; c) Sweeney, M.J., Davis, F.A., Gutowski, G.E., Hamill, R.L., Hoffman, D.H., Poore, G.A., *Cancer Res.* **1973**, 33, 2619; d) Ohnuma, T., Roboz, J., Shapiro, M., Holland, J.F., *Cancer Res.* **1977**, 37, 2043.

6) a) Just, G., Kim, S., *Tetrahedron Lett.* **1976**, 1063; b) Revankar, G.R., Solan, V.C., Robins, R.K., Witkowski, J.T., *Nucl. Acids Res. Symp. Ser.* **1981**, 9, 65;

c) Poonian, M.S., Nowoswiat, E.F., *J. Org. Chem.* **1980**, 45, 203; d) Ferris, J.P., Badesha, S.S., Ren, W.Y., Huang, H.C., Sorcek, R.J., *J. Chem. Soc. Chem. Comm.* **1981**, 110; e) Bimwala, R.M., Vogel, P., *Helv. Chim. Acta* **1989**, 72, 1825; f) Sauer, D.R., Schneller, S.W., *Nucleosides and Nucleotides* **1989**, 8, 1079; g) Kang, Y., Larson, S.B., Robins, R.K., Revankar, G.R., *J. Med. Chem.* **1989**, 32, 1547; h) Sauer, D.R., Schneller, S.W., *J. Org. Chem.* **1990**, 55, 5535; i) Franchetti, P., Cristalli, G., Grifantini, M., Cappellacci, L., Vittori, S., Nocentini, G., *J. Med. Chem.* **1990**, 33, 2849; j) Bergstrom, D.E., Zhang, P., *Tetrahedron Lett.* **1991**, 32, 6485; k) Sauer, D.R., Schneller, S.W., *Synthesis* **1991**, 747; l) Kovács, L., Herczegh, P., Batta, G., Farkas, I., *Tetrahedron* **1991**, 47, 5539; m) Kovács, L., Herczegh, P., Batta, G., Farkas, I., *Tetrahedron* **1991**, 47, 5549; n) Buchanan, J.G., Jumaah, A.O., Kerr, G., Talekar, R.R., Wightman, R.H., *J. Chem. Soc. Perkin Trans I* **1991**, 1077; o) Deceuninck, J.A., Verschave, P., Buffel, D.K., Tudonda, M., Hoornaert, G.J., *Nucleic Acid Chem.* part 4, (Towsend, L.B., Tipson, R.S., Eds.) Wiley interscience, **1991**, p 144; p) Buffel, D.K., Simons, B.P., Deceuninck , J.A., Hoornaert, G.J., *Nucleic Acid Chem.* part 4, (Towsend, L.B., Tipson, R.S., Eds.) Wiley interscience, **1991**, p 155; q) Ignacio, A.J., Garcia-Lopez, M.T., De Ias Heras, F.G., Mendez-Castrillon, P.P., *Nucleic Acid Chem.* part 4, (Towsend, L.B., Tipson, R.S., Eds.) Wiley interscience, **1991**, p 163; r) Kini, G.D., Petrie, C.R., Robins, R.K., *Nucleic Acid Chem.* **1991**, p 167.

7) a) Huybrechts, L., Buffel, D., Hoornaert, G., *Tetrahedron* **1984**, 40, 2479; b) Deceuninck, J., Buffel, D., Hoornaert, G., *Tetrahedron Letters* **1980**, 21, 3613.

8) For a review on formycin and formycin B, see ref. 3a) p.354 ,3b) p.169.

9) a) Acton, E.M., Ryan, K.J., Goodman, L., *J. Chem. Soc. Chem. Comm.* **1970**, 313; b) Acton, E.M., Ryan, K.J., Henry, D.W., Goodman, L., *J. Chem. Soc. Chem. Comm.* **1971**, 986; c) Kalvoda, L., *Coll. Czech. Chem. Comm.* **1978**, 43, 1431; d) Buchanan, J.G., Edgar, A.R., Hutchison, R.J., Stobie, A., Wightman, R., *J. Chem. Soc. Chem. Comm.* **1980**, 237; e) Buchanan, J.G., Edgar, A.R., Hutchison, R.J., Stobie, A., Wightman, R., *Can. J. Chem.* **1980**, 58, 2624; f) Gasparini, F., Vogel, P., *Helv. Chim. Acta* **1989**, 72, 271; g) El Khadem, H.S., Kawai, J., Swartz, D.L., *Carbohydr. Res.* **1989**, 189, 149; h) Dennin, F., Rousseaux, O., Blondeau, D., Sliwa, H., *J. Heterocycl. Chem.* **1989**, 26, 991; i) Girgis, N.S., Michael, M.A., Smee, D.F., Alaghamandan, H.A., Robins, R.K., Cottam, H.B., *J. Med. Chem.* **1990**, 33, 2750; j) Jeanneret, V., Gasparini, F., Péchy, P., Vogel, P., *Tetrahedron* **1992**, 48, 10637.

10) ref. 3b) p.172

11) ref. 3a) p. 381

12) a) Lim, M.I., Klein, R.S., Fox, J.J., *Tetrahedron Letters* **1980**, 21, 1013; b) Lim, M.I., Klein, R.S., *Tetrahedron Letters* 1981, 2, 25;c) Ren, W.Y., Lim, M.I., Otter, B.A., Klein, R.S., *J. Org. Chem.* **1982**, 47, 4633.

13) Buffel, D., Simons, B., Deceuninck, J., Hoornaert, G., *J. Org. Chem.* **1984**, 49, 2165.

14) Howe, R.K., Franz, J.E., *J. Org. Chem.* **1978**, 43, 3742.

15) Sustmann, R., Huisgen, R., Huber, H., *Chem. Ber.* **1967**, 100, 1802.

16) Adams, A., Slack, R., *J. Chem. Soc.* **1959**, 3061.

17) Weeler, O., Rosado, O., *The Chemistry of Amides* (Patai, S., Zabicki, J. Eds.) Interscience Publishers, London **1970**, p. 355.

18) Shealey, Y.F., O'Dell, C.A., *Nucleic Acid Chem.* part 1, (Townsend, L.B., Tipson, R.S., Eds.), Wiley Interscience **1978**, p 161.

19) Wasylishen, R.J., Clem, T.R., Becker, E.D., *Can. J. Chem.* **1975**, 53, 596.

20) Abrahams, R.J., Loftus, P. *Proton and Carbon-13 NMR Spectroscopy*, Heyden & Son, London, **1978**, p. 28.

21) Feeney, J., Partington, P., Roberts, G.C.K., *J. Magn. Res.* **1974**, 13, 268.

22) Wallis, E.S., Lane, J.F., *Org. Reactions* **1959**, 3, 267.

23) Holland, A., Slack, R., Warren, T.F., Buttimore, D., *J. Chem. Soc.* **1959**, 7277.

24) Imbach, J.L., Kam, B.L., *J. Carbohydr., Nucleosides & Nucleotides* **1974**, 1, 271.

25) Mc Coss, M., Robins, M.J., Rayner, B., Imbach, J.L., *Carbohydr. Res.* **1977**, 59, 575.

26) a) Horton, J.K., Stevens, M.G., *J. Chem. Soc. Perkin Trans I* **1981**, 1433; Goerdeler, J., Rögler, N., *Chem. Ber.* **1970**, 103, 112.

27) Montero, J.L., Tapiero, C., Imbach, J.L., *Nucleic Acid Chem.* part 2 (Townsend L.B., Tipson, R.S., Eds.), Wiley Interscience **1978**, p 645.

28) a) Roger, R., Neilson, D.G., *Chem. Rev.* **1961**, 61, 179; b) Hechelhammer, W., *Patentschrift B.R.D.* **1956**, 948,.973.

29) Pouchert, C.J., Campbell, J.R. *"The Aldrich Library of NMR spectra"*, Aldrich Chem. Co. Inc. Milwaukee **1974**, 9, 119.

30) Davies, D.B., Danyluk, S.S., *Biochemistry* **1974**, 13, 4417.

31) a) Altona, C., Sundaralingam, M., *J. Am. Chem. Soc.* **1972**, 94, 8205; Altona, C., Sundaralingam, M., *J. Am. Chem. Soc.* **1973**, 95, 2333.

32) Tran-Dinh, S., Neumann, J.-M., Thiéry, J.-M., Huynh-Dinh, T., Igolen, J., Guschlbauer, W. *J. Am. Chem. Soc.* **1977**, 99, 3267.

33) Haasnoot, C., de Leeuw, F., de Leeuw, H., Altona, C., *Recl. Trav. Chim. Pays-Bas* **1979**, 98, 576.

Received 7/18/93
Accepted 11/11/93