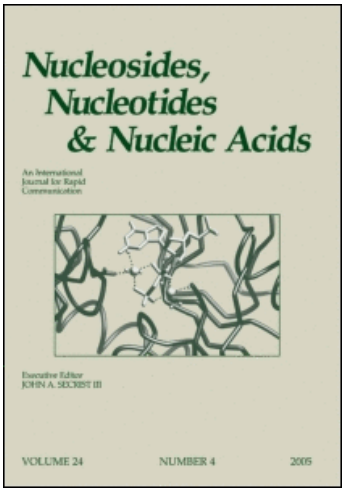


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biological Evaluation of Some Acyclic Pyridine C-Nucleosides. Part Two

Johan Van hemel[a]; Eddy L. Esmans[a]; Alex De Groot[b]; Roger A. Dommisse[b]; Jan M. Balzarini[c]; Erik D. De Clercq[c]
[a] Nucleoside Research Unit, University of Antwerp (RUCA), Antwerp, BELGIUM [b] NMR Research Unit, University of Antwerp (RUCA), Antwerp, BELGIUM [c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, BELGIUM

To cite this Article Van hemel, Johan , Esmans, Eddy L. , De Groot, Alex , Dommisse, Roger A. , Balzarini, Jan M. and De Clercq, Erik D.(1996) 'Synthesis and Biological Evaluation of Some Acyclic Pyridine C-Nucleosides. Part Two', Nucleosides, Nucleotides and Nucleic Acids, 15: 6, 1203 — 1221
To link to this Article: DOI: 10.1080/07328319608007388
URL: http://dx.doi.org/10.1080/07328319608007388

NUCLEOSIDES & NUCLEOTIDES, 15(6), 1203-1221 (1996)

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME ACYCLIC PYRIDINE C-NUCLEOSIDES. PART TWO.

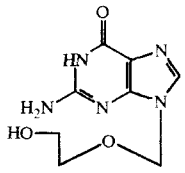
Johan Van hemel*[1], Eddy L. Esmans[1], Alex De Groot[2], Roger A. Dommisse[2], Jan M. Balzarini[3] and Erik D. De Clercq[3].
Nucleoside Research Unit[1] and NMR Research Unit[2].
University of Antwerp (RUCA),
Groenenborgerlaan 171, B-2020 Antwerp, BELGIUM.
Rega Institute for Medical Research[3]
Katholieke Universiteit Leuven,
Minderbroedersstraat 10, B-3000 Leuven, BELGIUM.

ABSTRACT.

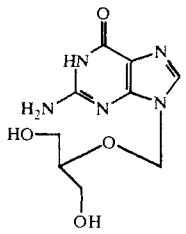
3-Bromo-5-(2-hydroxyethylthiomethyl)pyridine (7) was synthesized by reaction of 3-bromo-5-chloromethylpyridine hydrochloride (6) with the mono sodium salt of 2-mercaptoethanol. 3-Bromo-5-hydroxymethylpyridine (10) was, after protection as a silyl ether, converted to the 3-carboxy analogue using BuLi and CO_2. After deprotection with NH_4F, the alcohol function was chlorinated using $SOCl_2$. Finally, attachment of the acyclic chain and ammonolysis gave the acyclic nicotinamide nucleosides. Treatment of the latter compounds with Lawesson's reagent gave the thioamide analogues.
All compounds were identified by NMR and DCI-MS. The acyclic pyridine C-nucleosides were evaluated against a series of tumor-cell lines and a variety of viruses. No marked biological activity was found.

I. INTRODUCTION.

The discovery that acyclic analogues of natural nucleosides, such as 9-(2-hydroxyethoxymethyl)guanine (acyclovir)(1) and 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine (DHPG, ganciclovir)(2) possess potent and selective antiherpes activity has led to extensive efforts towards the synthesis of compounds of this type[1].



1

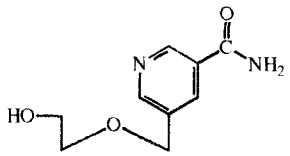


2

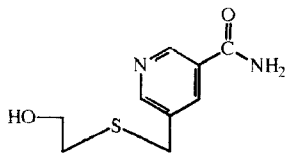
We have recently reported on the synthesis of some acyclovir-like acyclic pyridine C-nucleosides such as 3-carbamoyl-5-(2-hydroxyethoxymethyl)pyridine[2](3).

The substitution of sulfur for oxygen in order to obtain biologically active analogues has substantial precedent in medicinal chemistry. For instance, the thio analogue (5) was shown to exhibit a 5-fold lower *in vitro* antiherpes potency than acyclovir[3]. Another example is the 4'-thio-analogue of the antibiotic nucleoside toyocamycin which retains antibiotic and antileukemic activity[4].

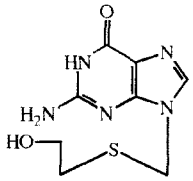
In order to study the *in vitro* SAR with pyridine C-nucleosides, we prepared 3-carbamoyl-5-(2-hydroxyethylthiomethyl)pyridine (4) and some analogues, which are the side-chain sulfur analogues of 3.



3



4



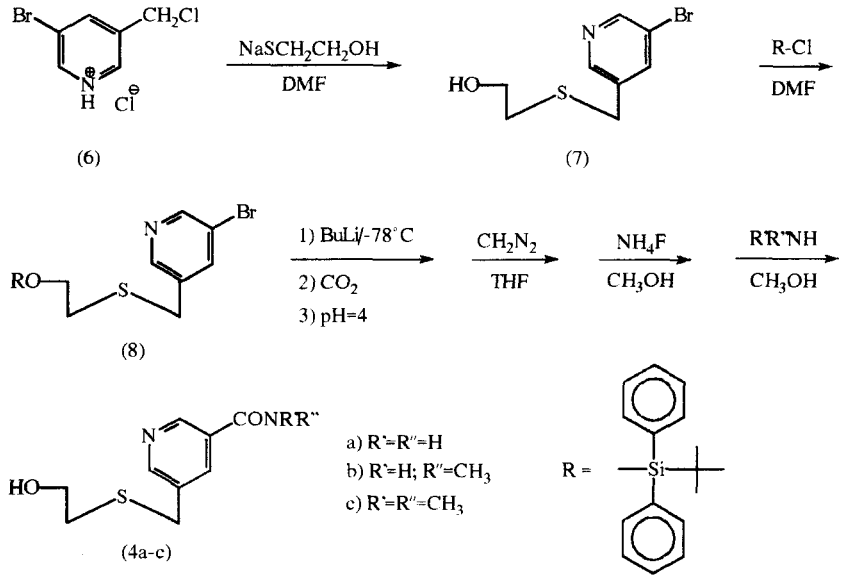
5

The synthesis and biological activity of these acyclic nucleosides are described herein.

II. RESULTS AND DISCUSSION.

II.a. AMIDES.

The synthesis of 4 was originally planned to proceed similarly as for compound 3[2] (SCHEME 1).

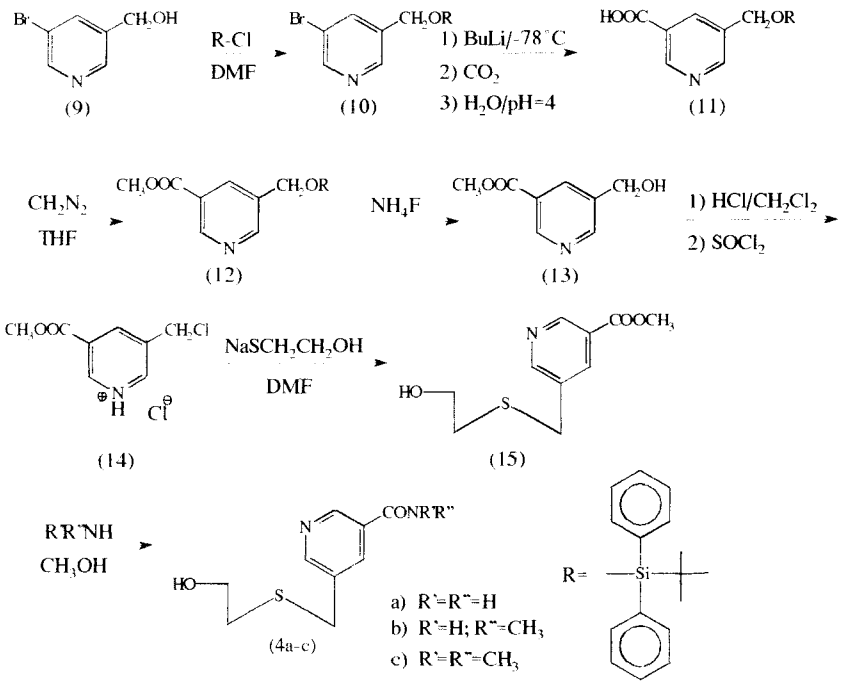


SCHEME 1.

The starting compound was 3-bromo-5-chloromethylpyridine hydrochloride (6), which was treated with 2.2 eq. of the mono sodium salt of 2-mercaptoethanol in DMF. After purification by column chromatography (CC), 3-bromo-5-(2-hydroxyethylthiomethyl)pyridine (7) was obtained in 85% yield. Prior to the lithiation step, the hydroxyl function was protected by a *tert*-butyldiphenylsilyl moiety. However, reaction of 8 with BuLi at -78°C, followed by treatment of the intermediate lithiopyridine with CO_2 resulted in the formation of 3-(2-hydroxy-ethylthiomethyl)pyridine instead of the carboxylic acid. This was probably due to the acidic protons of the CH_2-group between the sulfur atom and the pyridine moiety.

An attempt to deprotonate this CH_2-group by LDA prior to treatment with BuLi did not solve the problem.

To avoid these difficulties, the lithiation step should precede the introduction of the acyclic side chain. This alternative route is depicted in SCHEME 2.



SCHEME 2.

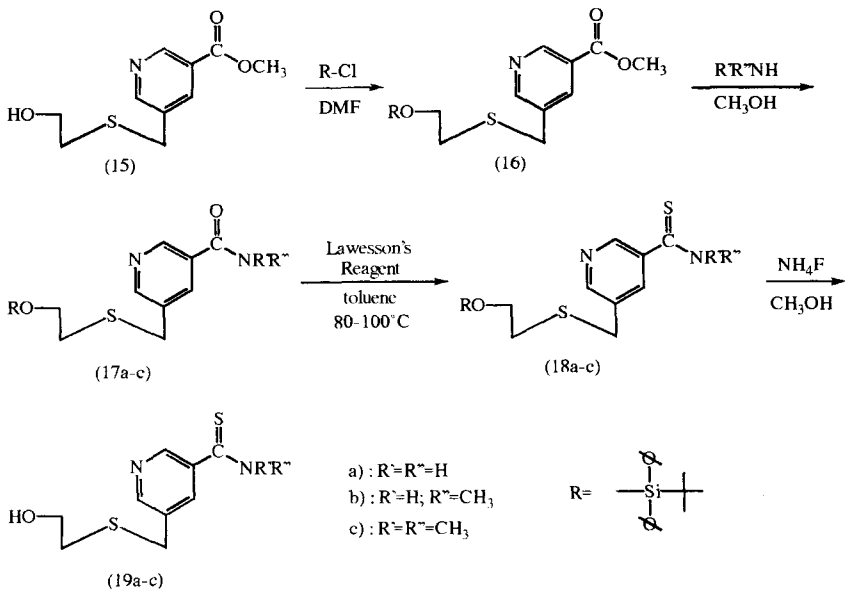
The alcohol function of 9 was protected as a silylether by reaction with *tert*-butyldiphenylsilyl chloride in DMF. 3-Bromo-5-*tert*-butyldiphenylsilyloxymethyl-pyridine (10) was obtained as a pale yellow oil in 90% yield. Treatment of compound 10 with 1.2 eq. BuLi in THF at -78°C, followed by reaction of the lithio compound with freshly prepared dry ice resulted in the formation of the carboxylic acid 11. This was, after adjusting the pH to 4, isolated by extraction with CH_2Cl_2. The carboxylic acid was then esterified with CH_2N_2, giving 3-methoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethylpyridine (12), after purification by CC, as a pale yellow oil. Removal of the silyl protecting group was accomplished with NH_4F in CH_3OH[5] instead of TBAF. NH_4F not only gave higher yields, but could also be removed much more easily after reaction than TBAF.

Chlorination of 5-hydroxymethyl-3-methoxycarbonylpyridine (13) with $SOCl_2$ gave the chloromethyl derivative 14 as a pale yellow solid in 76% yield. This product was, without further purification, reacted with 2.2 eq. of the mono sodium salt of 2-mercaptoethanol in DMF. Purification by CC resulted in the formation of the acyclic compound 15 as a white solid (84%).

Finally, an ammonolysis reaction converted 5-(2-hydroxyethylthiomethyl)pyridine (15) into the desired amide nucleosides 4a-c.

II.b. THIOAMIDES.

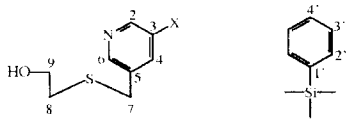
The thioamide nucleosides (19a-c) were synthesized using the same procedure as we reported earlier[2], i.e. reaction of 15 with *tert*-butyldiphenylsilylchloride to the protected ester 16, followed by an ammonolysis reaction which generated the amides 17a-c. These were then treated with Lawesson's reagent in toluene at elevated temperature (80°C and 100°C), resulting in the thioamides 18a-c. After removal of the silyl protecting group with NH_4F, the thioamides 19a-c were obtained as yellow products.



III. STRUCTURE IDENTIFICATION BY NMR.

All compounds were identified using 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectroscopy. The spectra were recorded in $CDCl_3$ or CD_3OD solutions, using the residual solvent signal [$CDCl_3$:δ = 77.00 ppm (^{13}C) and 7.3 ppm (^{1}H); CD_3OD : δ = 49.00 ppm (^{13}C) and 3.3 ppm (^{1}H)] or TMS as internal reference.

Basic numbering of our compounds was done as follows:



The carbon atoms of the acyclic chain were assigned by 2D HETCOR spectroscopy. Other assignments were accomplished using previously published results[2].

IV. BIOLOGICAL STUDIES.

Compounds 7, 4a-c, and 19a-c were evaluated for their cytostatic activity against murine leukemia L1210 cells and human T-lymphocyte Molt 4F and CEM cells. Only compounds 7 and 19b showed some cytostatic activity at a 50% inhibitory concentration (IC_{50}) of 100-200 µg/ml, as shown in TABLE 3 (5-fluoro-2'deoxyuridine (FdUrd) is given for comparison).

All above mentioned nucleosides were also evaluated for their inhibitory effects on the replication of a number of viruses, including herpes simplex virus type 1 (HSV-1)(strain KOS) and HSV-2 (strain G), vaccinia virus, vesicular stomatitis virus (VSV), thymidine kinase-deficient (TK^-) strains of HSV-1 (B2006 and VMW1837) in E_6SM cell cultures; VSV, Coxsackie virus B4 and polio virus-1 in HeLa cell cultures; parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Semliki forest virus in Vero cell cultures. No antiviral activity was noted at concentrations up to 400 µg/ml. Neither anti-HIV-1 (strain IIIb) nor anti-HIV-2 (strain ROD) activity was observed in MT-4 cells (IC_{50}>200 µg/ml).

The minimum cytotoxic concentration (i.e. the compound concentration required

TABLE 1 : ^{13}C-NMR data of the amides and the thioamides : δ-values in ppm.

	7	4a	4b	4c	19a	19b	19c
Solvent	$CDCl_3$	CD_3OD	CD_3OD	CD_3OD	CD_3OD	CD_3OD	CD_3OD
Reference	Solvent	Solvent	Solvent	Solvent	Solvent	Solvent	Solvent
C-2	149.33	147.51	147.43	146.85	146.90	148.68	145.51
C-3	120.75	131.34	131.70	133.57	137.37	138.90	141.04
C-4	139.10	137.91	137.03	136.83	136.84	136.77	135.40
C-5	136.09	137.32	137.03	137.02	136.44	136.52	136.62
C-6	147.74	152.64	152.84	151.54	152.29	151.74	150.04
C-7	32.67	33.74	33.75	33.69	33.79	33.77	33.74
C-8	34.85	34.82	34.78	34.78	34.87	34.82	34.81
C-9	60.92	62.34	62.34	62.37	62.34	62.33	62.37
C=O	-	169.47	168.11	170.53	-	-	-
C=S	-	-	-	-	200.60	197.05	197.70
NH-$\underline{CH_3}$	-	-	26.90	-	-	33.87	-
N-$\underline{(CH_3)_2}$	-	-	-	35.72; 39.90	-	-	43.57; 44.52

TABLE 2 : ^{1}H-NMR data of the amides and the thioamides : δ-values in ppm, coupling constants in Hz.

	7	4a	4b	4c	19a	19b	19c
Solvent	$CDCl_3$	CD_3OD	CD_3OD	CD_3OD	CD_3OD	CD_3OD	CD_3OD
Reference	Solvent	Solvent	Solvent	Solvent	Solvent	Solvent	Solvent
H-2	8.57 d	8.91 d	8.83 d	8.60 d	8.87 d	8.75 d	8.48 d
H-4	7.86 m	8.32 t	8.21 t	7.91 t	8.26 t	8.15 t	7.75 t
H-6	8.45 d	8.68 d	8.64 d	8.50 d	8.59 d	8.56 d	8.37 d
H-7	3.72 d	3.89 s	3.88 s	3.87 d	3.86 s	3.86 s	3.83 s
H-8	2.65 t	2.59 t	2.58 t	2.59 t	2.59 t	2.60 t	2.59 t
H-9	3.76 br	3.68 t	3.68 t	3.67 t	3.68 t	3.70 t	3.66 t
NH-$\underline{CH_3}$	-	-	2.94 s	-	-	3.26 s	-
N-$\underline{(CH_3)_2}$	-	-	-	3.02 s 3.13 s	-	-	3.20 s 3.58 s
J(2,4)	2.1	2.2	2.1	2.1	2.1	2.1	2.0
J(4,6)	2.0	2.0	2.0	2.0	2.0	2.0	2.1
J(4,7)	0.3	-	-	0.3	-	-	-
J(8,9)	6.0	6.6	6.6	6.6	6.6	6.6	6.6

TABLE 3.

Compound	L1210	Molt/4F	CEM
	IC_{50} (μg/ml)		
7	≥200	115 ± 47	96 ± 6.5
19b	157 ± 13	⟩200	≥200
FdUrd	0.001 ± 0.001	2.3 ± 0.5	0.003 ± 0.001

to cause a microscopically detectable alteration of normal E_6SM, Hela and Vero cell morphology) of all tested nucleosides was higher than 400 μg/ml.

V. EXPERIMENTAL.

General methods.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz Varian Unity 400 spectrometer. DCI-mass spectra were run on a Ribermag 10-10B 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, 0.25mm). Preparative centrifugal circular thin layer chromatography (CCTLC) was carried out on a Chromatotron® (Harrison Research, Palo Alto, CA). Stationary phase: Kieselgel 60 PF_{254} gipshaltig, Merck, layer thickness 2 mm, flow rate 5 ml/min. Column chromatography employed Merck silica gel (Kieselgel 60, 230-400 Mesh ASTM).

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

Reactions involving organometallic reagents were performed in oven-dried glassware under N_2-atmosphere. THF was dried by distillation from sodium/benzophenone ketyl prior to use. DMF was dried by distillation from

CaH_2 under reduced pressure. 5-Bromonicotinic acid, BuLi (1.6 M in hexane), NH_4F, 2-mercaptoethanol, Lawesson's reagent and *tert*-butyldiphenylsilylchloride were purchased from Janssen Chimica (Beerse, Belgium).

The cytostatic and antiviral assays were carried out according to previously published procedures[6,7,8,9].

Synthesis.

3-Bromo-5-(2-hydroxyethylthiomethyl)pyridine (7).

In a flask of 50 ml, 15 ml 2-mercaptoethanol was stirred with 0.83 g (36.5 mmol) Na-metal until H_2-formation ceased.

A three necked flask of 100 ml, equipped with a $CaCl_2$ tube, was filled with 20 ml dry DMF. Then 8.55 ml (2.2 eq.) of the above described $NaSCH_2CH_2OH/HSCH_2CH_2OH$-mixture was added. To this solution, 6 (2.3 g, 9.5 mmol) dissolved in some dry DMF was added dropwise, and the whole mixture was stirred at room temperature and under N_2-atmosphere for 24 hours. After evaporation of the solvent the obtained yellow oil was suspended in H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure and the obtained oil was purified by a column chromatography (CC)(20cm x 4cm I.D.) on silica gel (eluting with CH_2Cl_2/CH_3OH (90:10), R_f=0.61).This gave 3-bromo-5-(2-hydroxyethylthiomethyl)-pyridine (7) as a colorless oil (2.0 g, 85%).

DCI-mass spectrometry (NH_3) : m/z = 248 ($[MH]^+(^{79}Br)$, 100%).

Elemental analysis for $C_8H_{10}BrNOS$: calc. C, 38.72%; H, 4.06%; Br, 32.20%; N, 5.64%; S 12.92%. Found : C, 38.51%; H, 4.15%; Br, 31.99%; N, 5.60%; S, 12.85%.

3-Bromo-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)pyridine (8).

3-Bromo-5-(2-hydroxyethylthiomethyl)pyridine (7) (1.77 g, 7.14 mmol) was dissolved in dry DMF (50 ml), and *tert*-butyldiphenylsilyl chloride (2.16 g, 2.04 ml, 1.1 eq.) and imidazole (1.07 g, 2.2 eq.) were added. The solution was stirred at room temperature for 6 hours, after which it was poured in a saturated $NaHCO_3$ solution. This solution was then extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$. After evaporation of the solvent the obtained oil was purified by CC (30cm x 4cm I.D.)

on silica gel (eluting with hexane/EtOAc (80:20), R_f=0.55) and compound 8 was obtained as a colorless oil (3.0 g, 86%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 149.51 (C-2), 148.01 (C-6), 138.75 (C-4), 136.18 (C-5), 135.55 (C-2'), 133.39 (C-1'), 129.77 (C-4'), 127.73 (C-3'), 120.66 (C-3), 63.65 (C-9), 33.82 (C-8), 33.18 (C-7), 26.86 (-C-($\underline{CH_3}$)$_3$), 19.17 (-$\underline{C}$-(CH_3)$_3$).

^{1}H-NMR ($CDCl_3$, TMS) : δ 8.54 (1H, d, J=2.3 Hz, H-2), 8.36 (1H, d, J=1.8 Hz, H-6), 7.77 (1H, t, H-4), 7.65-7.69 (4H, m, H-2'), 7.36-7.46 (6H, m, H-3' and H-4'), 3.80 (2H, t, J=6.6 Hz, H-9), 3.62 (2H, s, H-7), 2.59 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-($\underline{CH_3}$)$_3$).

DCI-mass spectrometry (NH_3) : m/z = 486 ([MH]$^+$ (^{79}Br), 100%).

Elemental analysis for $C_{24}H_{28}BrNOSSi$: calc. C, 59.25%; H, 5.80%; Br, 16.40%; N, 2.88%; S, 6.59%. Found : C, 59.16%; H, 6.02%; Br, 16.31%; N, 3.05%; S, 6.31%.

3-Bromo-5-*tert*-butyldiphenylsilyloxymethylpyridine (10).

A mixture of 9 (2.04 g, 10.85 mmol), *tert*-butyldiphenylsilyl chloride (3.26 g, 3.1 ml, 1.1 eq.) and imidazole (1.93 g, 2.2 eq.) in dry DMF (50 ml), was stirred for 6 hours at room temperature. Then the mixture was poured into a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. The obtained oil was purified by CC (25cm x 4cm I.D.) on silica gel (eluting with hexane/EtOAc (80:20), R_f=0.68).

3-Bromo-5-*tert*-butyldiphenylsilyloxymethylpyridine (10) was obtained as a colorless oil (4.14 g, 90%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 149.38 (C-2), 145.94 (C-6), 138.16 (C-5), 136.69 (C-4), 135.51 (C-2'), 132.84 (C-1'), 129.99 (C-4'), 127.88 (C-3'), 120.66 (C-3), 62.81 (-$\underline{CH_2}$-), 26.83 (-C-($\underline{CH_3}$)$_3$), 19.26 (-$\underline{C}$-(CH_3)$_3$).

^{1}H-NMR ($CDCl_3$, solv. ref.) : δ 8.57 (1H, d, J=2.3 Hz, H-2), 8.45 (1H, d, J=1.8 Hz, H-6), 7.80 (1H, t, J=2.2 Hz, H-4), 7.66-7.70 (4H, m, H-2'), 7.36-7.47 (6H, m, H-3' and H-4'), 4.75 (2H, d, J=0.8 Hz, -$\underline{CH_2}$-), 1.10 (9H, s, -C-($\underline{CH_3}$)$_3$).

DCI-mass spectrometry (NH_3) : m/z = 426 ([MH]$^+$(^{79}Br), 100%).

Elemental analysis for $C_{22}H_{24}BrNOSi$: calc. C, 61.97%; H, 5.67%; Br, 18.74%; N, 3.28%. Found : C, 61.84%; H, 5.53%; Br, 18.81%; N, 3.57%.

5-*tert*-Butyldiphenylsilyloxyomethyl-3-methoxycarbonylpyridine (12).

a) A three necked flask of 100 ml, equipped with a dropping funnel, $CaCl_2$ tube and dry N_2 inlet system, was filled with 10 (1.04 g, 2.44 mmol) dissolved in 80 ml dry THF. The solution was cooled in a CO_2/acetone bath to -78 °C, and 1.8 ml BuLi (1.2 eq.) was added while stirring. After 5 minutes the contents were poured on a large excess of dry ice (200 g). After evaporation of the CO_2, the reaction mixture was evaporated in vacuo to dryness and the residue was dissolved in H_2O. This aqueous solution was acidified to pH=4 (HCl) and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to obtain 11.

DCI-mass spectrometry (NH_3) : m/z = 392 ($[MH]^+$, 100%).

b) The carboxylic acid (11) (2.7 g, 6.89 mmol) was dissolved in THF, cooled to 0 °C and an excess (2.5 eq.) of ethereal diazomethane (10 g Diazogen in 90 ml ether added to 12 ml of ethanol , 2.3 g KOH and 3.6 ml H_2O) was added. After evaporation of the solvent, the residue was purified by CC (25cm x 4cm I.D.) on silica gel (eluting with CH_2Cl_2/CH_3OH (99:1), R_f=0.45) and 12 was collected as a colorless oil (35-50%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 165.81 (C=O), 151.71 (C-6), 149.54 (C-2), 136.18 (C-5), 135.52 (C-2'), 134.95 (C-4), 132.93 (C-1'), 129.93 (C-4'), 127.85 (C-3'), 125.69 (C-3), 63.16 (-$\underline{CH_2}$-), 52.31 (-O-$\underline{CH_3}$), 26.83 (-C-($\underline{CH_3}$)$_3$), 19.26 (-$\underline{C}$-(CH_3)$_3$).

^{1}H-NMR ($CDCl_3$, solv. ref.) : δ 9.12 (1H, d, J=2.0 Hz, H-2), 8.74 (1H, d, J=2.1 Hz, H-6), 8.24 (1H, t, J=2.1 Hz, H-4), 7.67-7.69 (4H, m, H-2'), 7.38-7.48 (6H, m, H-3' and H-4'), 4.82 (2H, d, J=0.6 Hz, -$\underline{CH_2}$-), 3.96 (3H, s, -O-$\underline{CH_3}$), 1.12 (9H, s, -C-($\underline{CH_3}$)$_3$).

DCI-mass spectrometry (NH_3) : m/z = 406 ($[MH]^+$, 100%).

Elemental analysis for $C_{24}H_{27}NO_3Si$: calc. C, 71.08%; H, 6.71%, N, 3.45%. Found : C, 71.02%; H, 6.49%; N, 3.36%.

5-Hydroxymethyl-3-methoxycarbonylpyridine (13).

A solution of 5-*tert*-butyldiphenylsilyloxymethyl-3-methoxycarbonylpyridine (12)(95 mg, 0.234 mmol) and NH_4F (65 mg, 2.57 mmol) in methanol (2 ml) was stirred in an oil bath at 60°C for 3 hours. The solvent was removed under reduced pressure and the residue was purified by CC (6cm x 2cm I.D.) on silica gel (eluting with CH_2Cl_2/CH_3OH (90:10), R_f=0.51). This gave the obtained product 13 as a white solid (90%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 165.61 (C=O), 151.79 (C-6), 149.54 (C-2), 136.60 (C-

5), 135.68 (C-4), 125.97 (C-3), 61.92 (-$\underline{CH_2}$-), 52.39 (-O$\underline{CH_3}$).

^{1}H-NMR ($CDCl_3$, solv. ref.) : δ 9.04 (1H, d, J=2.0 Hz, H-2), 8.69 (1H, d, J=2.1 Hz, H-6), 8.30 (1H, t, J=2.1 Hz, H-4), 4.78 (2H, s, -$\underline{CH_2}$-), 3.93 (3H, s, -O$\underline{CH_3}$).

DCI-mass spectrometry (NH_3) : m/z = 168 ([MH]$^+$, 100%).

Elemental analysis for $C_8H_9NO_3$: calc. C, 57.48%; H, 5.43%; N, 8.38%. Found : C, 57.61%; H, 5.40%; N, 8.47%.

5-Chloromethyl-3-methoxycarbonylpyridine hydrochloride (14).

The alcohol 13 (350 mg, 2.09 mmol) was dissolved in 30 ml dry CH_2Cl_2 saturated with HCl-gas. After 5 min stirring the solvent was evaporated. To this solid residue 5 ml $SOCl_2$ was added at 0 °C after which the solvent was refluxed for 2 hours. After this period the solution was cooled to room temperature and evaporated to dryness. This gave 14 (350 mg, 1.58 mmol) as a pale yellow solid, which was used in the next reaction step without further purification. Yield (crude) : 76%.

DCI-mass spectrometry (NH_3) m/z = 186 ([MH]$^+$, 100%).

5-(2-Hydroxyethylthiomethyl)-3-methoxycarbonylpyridine (15).

5-(2-Hydroxyethylthiomethyl)-3-methoxycarbonylpyridine (15) was synthesized following the same procedure as for 7, i.e. by reaction of 14 (350 mg, 1.54 mmol) with 2.2 eq. of the mono sodium salt of 2-mercaptoethanol in dry DMF. The product 15 was purified by CC (20cm x 4cm I.D.) on silica gel (eluting with CH_2Cl_2/CH_3OH (95:5), R_f=0.32), yielding 15 as a white solid (84%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 165.47 (C=O), 153.44 (C-6), 149.41 (C-2), 137.24 (C-4), 134.17 (C-5), 125.98 (C-3), 60.84 (C-9), 52.39 (-O$\underline{CH_3}$), 34.44 (C-8), 32.90 (C-7).

^{1}H-NMR ($CDCl_3$, solv. ref.) : δ 9.06 (1H, d, J=2.0 Hz, H-2), 8.68 (1H, d, J=2.3 Hz, H-6), 8.26 (1H, t, J=2.1 Hz, H-4), 3.93 (3H, s, -O-$\underline{CH_3}$), 3.78 (2H, s, H-7), 3.73 (2H, t, J=6.0 Hz, H-9), 2.62 (2H, t, J=6.0 Hz, H-8).

DCI-mass spectrometry (NH_3) : m/z = 228 ([MH]$^+$, 100%).

Elemental analysis for $C_{10}H_{13}NO_3S$: calc. C, 52.85%; H, 5.77%; N, 6.16%; S 14.11%. Found : C, 52.72%; H, 5.94%; N, 6.35%; S, 13.97%.

3-Carbamoyl-5-(2-hydroxyethylthiomethyl)pyridine (4a), 3-(N-methylcarbamoyl)-5-(2-hydroxyethylthiomethyl)pyridine (4b) and 3-(N,N-dimethylcarbamoyl)-5-(2-hydroxyethylthiomethyl)pyridine (4c).

The methylester (15) (100 mg) was suspended in CH_3OH (50 ml), cooled to 0 °C and saturated with NH_3, CH_3NH_2 or $(CH_3)_2NH$. The mixture was then stirred at room temperature for 2 days. After evaporation of the solvent in vacuo, the residues were purified by CCTLC (CH_2Cl_2/CH_3OH (90:10)), yielding the amides 4a-c nearly quantitatively.

DCI-mass spectrometry (NH_3) : m/z = 213 (4a)([MH^+], 100%), m/z = 227 (4b)([MH^+] 100%) and m/z = 241 (4c)([MH^+], 100%).

Elemental analysis for $C_9H_{12}N_2O_2S$ (4a) : calc. C, 50.93%; H, 5.70%; N, 13.20%; S, 15.10%. Found : C, 50.78%; H, 5.55%; N, 12.96%; S, 15.21%.

Elemental analysis for $C_{10}H_{14}N_2O_2S$ (4b) : calc. C, 53.08%; H, 6.24%; N, 12.38%; S, 14.17%. Found : C, 53.19%; H, 6.02%; N, 12.13%; S, 14.29%.

Elemental analysis for $C_{11}H_{16}N_2O_2S$ (4c) : calc. C, 54.98%; H, 6.71%; N, 11.66%; S, 13.34%. Found : C, 54.83%; H, 6.64%; N, 11.82%; S, 13.18%.

5-(2-*tert*-Butyldiphenylsilyloxyethylthiomethyl)-3-methoxycarbonylpyridine (16).

5-(2-Hydroxyethylthiomethyl)-3-methoxycarbonylpyridine (15) (680 mg, 2.98 mmol) was dissolved in dry DMF (20 ml), and *tert*-butyldiphenylsilylchloride (0.89 g, 0.85 ml, 1.1 eq.) and imidazole (0.53 g, 2.2 eq.) were added. The solution was stirred at room temperature for 6 hours, after which it was poured into a saturated $NaHCO_3$ solution. This solution was then extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$. After evaporation of the solvent, the resulting oil was purified by column chromatography (10cm x 4cm I.D.) on silica gel (eluting with hexane/EtOAc (80:20), R_f=0.25). This gave 16 as a colorless oil (950 mg, 70%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 165.59 (C=O), 153.58 (C-6), 149.47 (C-2), 137.16 (C-4), 135.57 (C-2'), 134.59 (C-5), 133.40 (C-1'), 129.77 (C-4'), 127.73 (C-3'), 125.90 (C-3), 63.66 (C-9), 52.38 (-O$\underline{C}H_3$), 33.85 (C-8), 33.46 (C-7), 26.85 (-C-($\underline{C}H_3)_3$), 19.18 (-$\underline{C}$-$(CH_3)_3$).

^{1}H-NMR ($CDCl_3$, solv. ref.) : δ 9.09 (1H, d, J=2.1 Hz, H-2), 8.62 (1H, d, J=2.2 Hz, H-6), 8.23 (1H, t, J=2.1 Hz, H-4), 7.65-7.69 (4H, m, H-2'), 7.36-7.45 (6H, m, H-3' and H-

4'), 3.95 (3H, s, -O$\underline{CH_3}$), 3.80 (2H, t, J=6.6 Hz, H-9), 3.71 (2H, s, H-7), 2.60 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-$\underline{(CH_3)_3}$).

DCI-mass spectrometry (NH_3) : m/z = 466 ($[MH]^+$, 100%).

Elemental analysis for $C_{26}H_{31}NO_3SSi$: calc. C, 67.06%; H, 6.71%; N, 3.01%; S, 6.88%. Found : C, 66.95%; H, 6.59%; N, 3.08%; S, 7.00%.

3-Carbamoyl-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)pyridine (17a), 3-(N-methylcarbamoyl)-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)pyridine (17b) and 3-(N,N-dimethylcarbamoyl)-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)pyridine (17c).

The synthesis of the amides 17a-c was accomplished by the same method as described for 4a-c. The products were purified by CC (9cm x 4cm I.D.) on silica gel (eluting with CH_2Cl_2/CH_3OH (95:5)). The amides (17a-c) were obtained nearly quantitatively.

^{13}C-NMR.

(17a) : ($CDCl_3$, solv. ref.) : δ 167.17 (C=O), 152.79 (C-6), 146.93 (C-2), 135.55 (C-2'), 135.49 (C4), 134.65 (C-5), 133.42 (C-1'), 129.78 (C-4'), 128.96 (C-3), 127.74 (C-3'), 63.68 (C-9), 34.00 (C-8), 33.53 (C-7), 26.87 (-C-$\underline{(CH_3)_3}$), 19.20 (-$\underline{C}$-$(CH_3)_3$).

(17b) : ($CDCl_3$, solv. ref.) : δ 166.05 (C=O), 152.24 (C-6), 146.49 (C-2), 135.54 (C-2'), 135.02 (C-4), 134.52 (C-5), 133.44 (C-1'), 130.13 (C-3), 129.77 (C-4'), 127.73 (C-3'), 63.69 (C-9), 33.97 (C-8), 33.55 (C-7), 26.87 (-C-$\underline{(CH_3)_3}$ and -NH-$\underline{CH_3}$), 19.20 (-$\underline{C}$-$(CH_3)_3$).

(17c) : ($CDCl_3$, solv. ref.) : δ 168.76 (C=O), 150.69 (C-6), 146.62 (C-2), 135.55 (C-2'), 135.11 (C-1'), 134.26 (C-5), 133.40 (C-4), 131.91 (C-3), 129.78 (C-4'), 127.74 (C-3'), 63.63 (C-9), 39.46 and 35.41 (-N-$\underline{(CH_3)_2}$), 33.93 (C-8), 33.56 (C-7), 26.86 (-C-$\underline{(CH_3)_3}$), 19.18 (-$\underline{C}$-$(CH_3)_3$).

^{1}H-NMR.

(17a) : ($CDCl_3$, TMS) : δ 8.89 (1H, d, J=2.1 Hz, H-2), 8.59 (1H, d, J=2.1 Hz, H-6), 8.08 (1H, t, H-4), 7.65-7.68 (4H, m, H-2'), 7.36-7.44 (6H, m, H-3' and H-4'), 6.1 (2H, broad, -$\underline{NH_2}$), 3.81 (2H, t, J=6.6 Hz, H-9), 3.71 (2H, s, H-7), 2.60 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-$\underline{(CH_3)_3}$).

(17b) : ($CDCl_3$, TMS) : δ 8.83 (1H, d, J=2.0 Hz, H-2), 8.55 (1H, d, J=2.0 Hz, H-6), 8.01 (1H, t, H-4), 7.64-7.68 (4H, m, H-2'), 7.36-7.44 (6H, m, H-3' and H-4'), 6.3 (1H, broad, $\underline{NH}$-CH_3), 3.80 (2H, t, J=6.6 Hz, H-9), 3.70 (2H, s, H-7), 3.00 (3H, d, J=4.9 Hz, -

NH-$\underline{CH_3}$), 2.59 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-$\underline{(CH_3)_3}$).

(17c) : ($CDCl_3$, TMS) : δ 8.55 (1H, d, J=2.0 Hz, H-2), 8.49 (1H, d, J=2.3 Hz, H-6), 7.69 (1H, t, H-4), 7.65-7.69 (4H, m, H-2'), 7.36-7.44 (6H, m, H-3' and H-4'), 3.81 (2H, t, J=6.6 Hz, H-9), 3.68 (2H, s, H-7), 2.96 and 3.12 (6H, s, N-$\underline{(CH_3)_2}$), 2.61 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-$\underline{(CH_3)_3}$).

DCI-mass spectrometry (NH_3).

(17a) : m/z = 451 ($[MH]^+$, 100%).

(17b) : m/z = 465 ($[MH]^+$, 100%).

(17c) : m/z = 479 ($[MH]^+$, 100%).

Elemental analysis for $C_{25}H_{30}N_2O_2SSi$ (17a) : calc. C, 66.63%; H, 6.71%; N, 6.22%; S, 7.11%. Found : C, 66.48%; H, 6.65%; N, 6.27%; S, 7.02%.

Elemental analysis for $C_{26}H_{32}N_2O_2SSi$ (17b) : calc. C, 67.20%; H, 6.94%; N, 6.03%; S, 6.90%. Found : C, 66.97%; H, 6.89%; N, 6.14%; S, 6.73%.

Elemental analysis for $C_{27}H_{34}N_2O_2SSi$ (17c) : calc. C, 67.74%; H, 7.16%; N, 5.85%; S, 6.70%. Found : C, 67.61%; H, 7.08%; N, 6.00%; S, 6.55%.

3-Thiocarbamoyl-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)pyridine (18a).

To a solution of 17a (166 mg, 0.37 mmol) in toluene (3 ml) was added Lawesson's reagent (75 mg), and the mixture was stirred at 80 °C for 2 h. The solution was allowed to cool to room temperature, poured into a saturated $NaHCO_3$ solution (30 ml), and extracted with EtOAc (3 x 30 ml). The organic layers were washed with saturated $NaHCO_3$ solution (3 x 30 ml) and then with brine (3 x 30 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by preparative CCTLC, eluting with CH_2Cl_2/CH_3OH ((95:5), R_f=0.47) to give the desired thioamide 18a as a yellow foam (55%).

^{13}C-NMR ($CDCl_3$, solv. ref.) : δ 199.70 (C=S), 152.48 (C-6), 145.33 (C-2), 135.57 (C-2'), 135.30 (C-4), 134.78 (C-3), 134.41 (C-5), 133.45 (C-1'), 129.81 (C-4'), 127.77 (C-3'), 63.76 (C-9), 34.04 (C-8), 33.53 (C-7), 26.90 (-C-$\underline{(CH_3)_3}$), 19.23 (-$\underline{C}$-$(CH_3)_3$).

^{1}H-NMR ($CDCl_3$, TMS) : δ 8.89 (1H, d, J=2.3 Hz, H-2), 8.56 (1H, d, J=2.1 Hz, H-6), 8.12 (1H, t, H-4), 7.65-7.69 (4H, m, H-2'), 7.35-7.46 (6H, m, H-3' and H-4'), 3.82 (2H, t, J=6.5 Hz, H-9), 3.71 (2H, s, H-7), 2.61 (2H, t, J=6.5 Hz, H-8), 1.06 (9H, s, -C-$\underline{(CH_3)_3}$).

DCI-mass spectrometry (NH_3) : m/z = 467 ($[MH]^+$, 21%), 433 ($[MH^+ - H_2S]$, 100%). Elemental analysis for $C_{25}H_{30}N_2OS_2Si$: calc. C, 64.34%; H, 6.48%; N, 6.00%; S, 13.74%. Found : C, 64.12%; H, 6.31%; N, 6.12%; S, 13.91%.

3-(N-methylthiocarbamoyl)-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)pyridine (18b) and 3-(N,N-dimethylthiocarbamoyl)-5-(2-*tert*-butyldiphenylsilyloxyethylthiomethyl)-pyridine (18c).

The thioamides 18b and 18c were synthesized by stirring a solution of 17b or 17c in toluene with Lawesson's reagent at 100 °C for 2 h. The obtained mixtures were then treated by the same procedure as described for the synthesis of 18a. The thioamides were purified by CC on silica gel (eluting with CH_2Cl_2/CH_3OH (98:2), R_f=0.24 (18b) and 0.35 (18c). This gave the thioamides 18b (85%) and 18c (66%) as a yellow foam.

^{13}C-NMR.

(18b) : ($CDCl_3$, solv. ref.) : δ 196.90 (C=S), 151.64 (C-6), 145.20 (C-2), 137.22 (C-3), 135.54 (C-2'), 135.05 (C-4), 134.33 (C-5), 133.45 (C-1'), 129.78 (C-4'), 127.76 (C-3'), 63.73 (C-9), 34.03 (C-8), 33.69 (NH-CH_3), 33.55 (C-7), 26.88 (-C-$(CH_3)_3$), 19.20 (-C-$(CH_3)_3$).

(18c) : ($CDCl_3$, solv. ref.) : δ 197.34 (C=S), 149.57 (C-6), 144.81 (C-2), 138.97 (C-3), 135.54 (C-2'), 134.14 (C-5), 133.89 (C-4), 133.42 (C-1'), 129.77 (C-4'), 127.74 (C-3'), 63.66 (C-9), 44.08 and 43.22 (N-$(CH_3)_2$), 34.04 (C-8), 33.59 (C-7), 26.87 (-C-$(CH_3)_3$), 19.19 (-C-$(CH_3)_3$).

^{1}H-NMR.

(18b) : ($CDCl_3$, TMS) : δ 8.73 (1H, d, J=2.3 Hz, H-2), 8.49 (1H, d, J=2.1 Hz, H-6), 8.01 (1H, t, H-4), 7.64-7.68 (4H, m, H-2'), 7.36-7.45 (6H, m, H-3' and H-4'), 3.81 (2H, t, J=6.6 Hz, H-9), 3.69 (2H, s, H-7), 3.32 (3H, d, J=4.9 Hz, NH-CH_3), 2.60 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-$(CH_3)_3$).

(18c) : ($CDCl_3$, TMS) : δ 8.43 (1H, d, J=2.1 Hz, H-2), 8.42 (1H, d, J=2.1 Hz, H-6), 7.65-7.69 (4H, m, H-2'), 7.60 (1H, t, H-4), 7.36-7.45 (6H, m, H-3' and H-4'), 3.81 (2H, t, J=6.6 Hz, H-9), 3.66 (2H, s, H-7), 3.58 and 3.13 (6H, s, N-$(CH_3)_2$), 2.62 (2H, t, J=6.6 Hz, H-8), 1.06 (9H, s, -C-$(CH_3)_3$).

DCI-mass spectrometry (NH_3).

(18b) : m/z = 481 ($[MH]^+$, 100%).

(18c) : m/z = 495 ([MH]$^+$, 100%).

Elemental analysis for $C_{26}H_{32}N_2OS_2Si$ (18b) : calc. C, 64.96%; H, 6.71%; N, 5.83%; S, 13.34%. Found : C, 64.39%; H, 6.65%; N, 5.70%; S, 13.35%.

Elemental analysis for $C_{27}H_{34}N_2OS_2Si$ (18c) : calc. C, 65.54%; H, 6.93%; N, 5.66%; S, 12.96%. Found : C, 65.70%; H, 6.98%; N, 5.47%; S, 13.31%.

3-Thiocarbamoyl-5-(2-hydroxyethylthiomethyl)pyridine (19a), 3-(N-methylthiocarbamoyl)-5-(2-hydroxyethylthiomethyl)pyridine (19b) and 3-(N,N-dimethylthiocarbamoyl)-5-(2-hydroxyethylthiomethyl)pyridine (19c).

Removal of the silyl protecting group was accomplished using the same procedure as described for the synthesis of 13. The products were purified by CC (eluting with CH_2Cl_2/CH_3OH (95:5), R_f=0.20 (19a) or CH_2Cl_2/CH_3OH (90:10), R_f=0.60 (19b) and R_f=0.63 (19c)).

Yields : (19a) : 76%.
(19b) : 66%.
(19c) : 90%.

DCI-mass spectrometry (NH_3).

(19a) : m/z = 229 ([MH]$^+$, 32%), 195 ([MH$^+$-H_2S], 100%).

(19b) : m/z = 243 ([MH]$^+$, 100%).

(19c) : m/z = 257 ([MH]$^+$, 100%).

Elemental analysis for $C_9H_{12}N_2OS_2$ (19a) : calc. C, 47.34%; H, 5.30%; N, 12.27%, S, 28.08%. Found : C, 47.11%; H, 5.42%; N, 12.39%; S, 27.92%.

Elemental analysis for $C_{10}H_{14}N_2OS_2$ (19b) : calc. C, 49.56%; H, 5.82%; N, 11.56%; S, 26.46%. Found : C, 49.71%; H, 5.64%; N, 11.38%; S, 26.67%.

Elemental analysis for $C_{11}H_{16}N_2OS_2$ (19c) : calc. C, 51.53%; H, 6.29%; N, 10.93%; S, 25.01%. Found : C, 51.38%; H, 6.07%; N, 10.88%; S, 25.14%.

ACKNOWLEDGEMENT

J. Van hemel is indebted to the I.W.O.N.L. for a grant. We thank J. Verreydt, J. Aerts, A. Van Lierde, F. De Meyer, A. Absillis and L. van Berckelaer for technical assistance. We are indebted to the Belgian N.F.W.O. (Nationaal Fonds voor Wetenschappelijk Onderzoek, project 3.3010.91), the Belgian F.G.W.O. (Fonds voor Geneeskundig

Wetenschappelijk Onderzoek, project 3.0180.95), the Belgian G.O.A. (Geconcerteerde Onderzoeksacties, project 95/5) and the Biomedical Research Programme of the European Community for financial support.

REFERENCES

1. C.K. Chu and S.J. Cutler; J. Heterocyclic Chem., 23, 289 (1986).

2. J. Van hemel, E.L. Esmans, F.C. Alderweireldt, R.A. Dommisse, A. De Groot, J. Balzarini and E. De Clercq; Nucleos. Nucleot., 13, 2345 (1994).

3. P.M. Keller, J.A. Fyfe, L. Beauchamp, C.M. Lubbers, P.A. Furman, H.J. Schaeffer, G.B. Elion; Biochem. Pharmacol., 15, 168 (1981).

4. M. Bobek, R.L. Whistler and A. Bloch; J. Med. Chem., 15, 168 (1972).

5. W. Zhang, M.J. Robins; Tetrahedron Lett., 33, 1177 (1992).

6. E. De Clercq, J. Balzarini, P.F. Torrence, M.P. Mertes, C.L. Schmidt, D. Shugar, P.J. Barr, A.S. Jones, G. Verhelst, R.T. Walker; Molec. Pharmacol., 19, 321 (1981).

7. E. De Clercq, J. Descamps, G. Verhelst, R.T. Walker, A.S. Jones, P.F. Torrence, D. Shugar; J. Infect. Dis., 141, 563 (1980).

8. E. De Clercq; Antimicrob. Agents Chemother., 28, 84 (1985).

9. J. Balzarini, M. Baba, R. Pauwels, P. Herdewijn, S.G. Wood, M.J. Robins, E. De Clercq; Molec. Pharmacol., 33, 243 (1988).

Received October 10, 1995
Accepted February 15, 1996