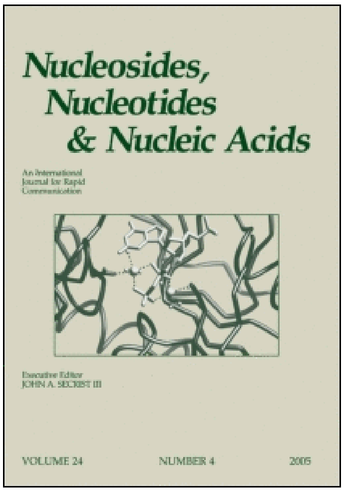

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Synthesis and Biological Evaluation of Phosphonate Derivatives of Some Acyclic Pyridine-C-Nucleosides

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SYNTHESIS AND BIOLOGICAL EVALUATION OF PHOSPHONATE DERIVATIVES OF SOME ACYCLIC PYRIDINE-C-NUCLEOSIDES.

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ABSTRACT.

The synthesis of phosphonomethoxy derivatives of some acyclic pyridine-C-nucleosides is described. Reaction of the acyclic nucleoside alkoxides with diethyl (tosyloxymethyl)phosphonate (5) led to the protected phosphonate derivatives. After eventual modification of the pyridine moiety, the ethyl protecting groups could be removed by treatment with bromotrimethylsilane. The phosphonates were evaluated against a series of tumor cell lines and a variety of viruses. Compared to the nucleoside analogues, they showed a slightly higher activity.

INTRODUCTION.

In recent years it has been shown that acyclic phosphonomethoxy analogues of nucleoside 5'-monophosphates can lead to potent and selective antiviral agents. Three series of the potent antiviral acyclic phosphonate nucleotide analogues are the 9-[2-(phosphonomethoxy)ethyl] (PME), (R)-9-[2-(phosphonylmethoxy)-propyl] [(R)-PMP] and (S)-9-[3-hydroxy-2-(phosphonomethoxy)-propyl] [(S)-

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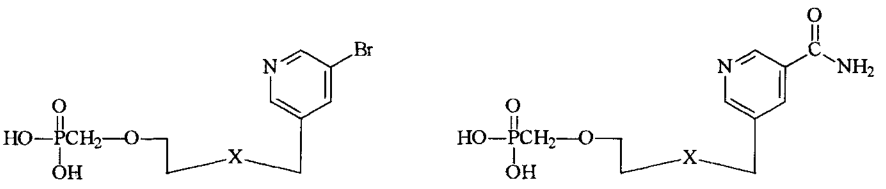
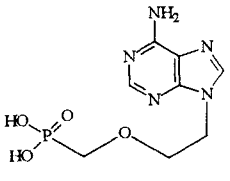


1 : X = O
2 : X = S

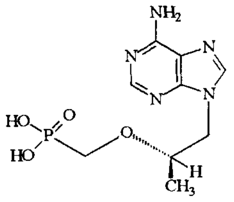
3 : X = O
4 : X = S

FIGURE 1.

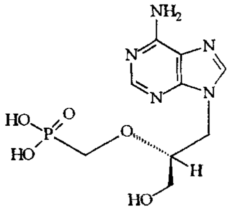
HPMP] series.[1,2,3] In the first series PMEA has activity against herpes viruses and retroviruses including HIV. In the second series, (R)-PMPA is exclusively active against retroviruses.[4] In the latter series (S)-HPMPA is a broad spectrum antiviral compound, and shows activity, besides HSV-1 and HSV-2, also against varicella-zoster virus (VZV), cytomegalovirus (CMV), adenoviruses, vaccinia virus and Moloney murine sarcoma virus (MSV).[5,6,7] Besides a potent activity against CMV, the corresponding cytosine derivative [(S)-HPMPC] has recently also been shown to display activity against HSV,[8] polyomaviruses[9] and papilloma.[10] For a recent overview on HPMPC and HPMPA, see Naesens *et al.*[11]



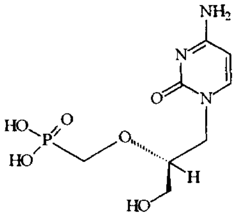
Adefovir
PMEA



(R)-PMPA



(S)-HPMPA



Cidofovir
(S)-HPMPC

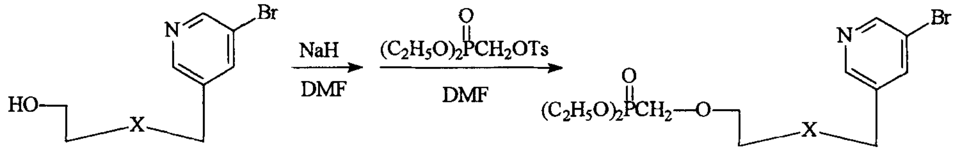
Since the activity of these phosphonate analogues is not dependent on an initial phosphorylation step by viral thymidine kinases (TKs), they are also active against TK-deficient mutants of HSV-1 which are resistant to acyclovir (ACV).[12] As part of our programme on the synthesis of acyclic pyridine-C-nucleosides,[13,14] we have now synthesized phosphonate derivatives of these nucleosides (FIGURE 1).

In this paper, we report on the synthesis and *in vitro* biological activity of these compounds.

RESULTS AND DISCUSSION.

The phosphonate group was introduced by reaction of the acyclic nucleoside alkoxide with an activated dialkyl hydroxymethylphosphonate. Diethyl (tosyloxymethyl)phosphonate (**5**) was used as the phosphonate reagent. The synthesis of **5** was carried out in a one-pot reaction as described by Bronson *et al.*[15]

A mixture of the bromo nucleoside (**6**, **7**), diethyl (tosyloxymethyl)phosphonate (**5**, 2 eq.) and NaH (2 eq.), dissolved in dry DMF, was stirred at room temperature for 3 hours under N_2-atmosphere. After working-up, the products were purified by column chromatography (CC) and circular centrifugal thin layer chromatography (CCTLC). This afforded the diethyl phosphonyl derivatives **8** and **9** in 30-39% yield.



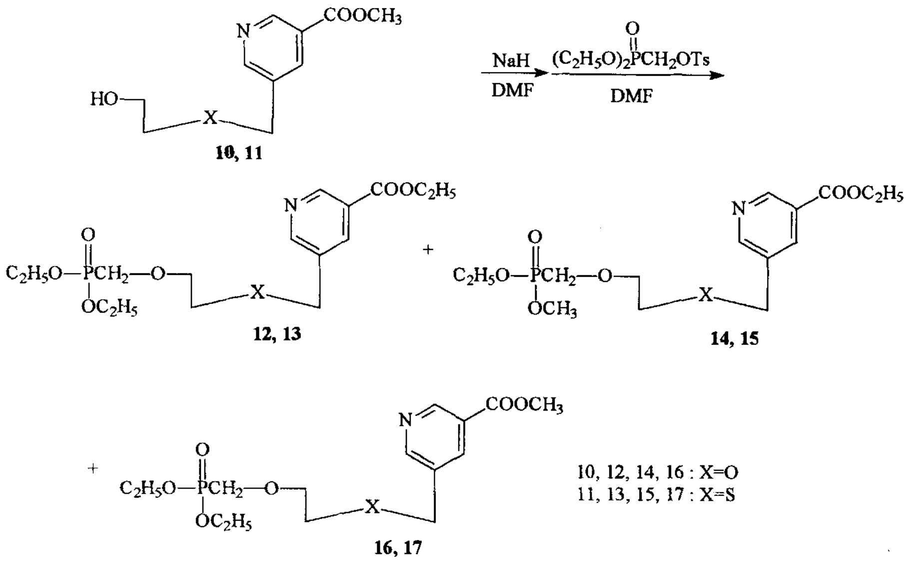
6, 7

8, 9

6, 8 : X=O
7, 9 : X=S

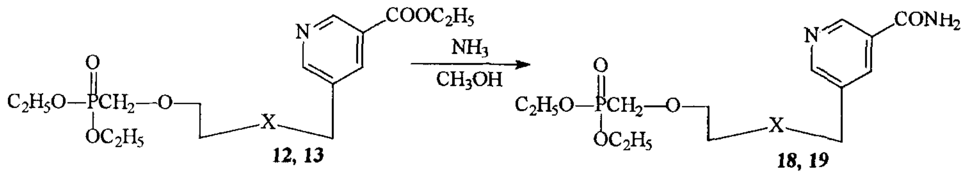
The coupling reaction of the ester derivatives **10** and **11** was carried out using the same procedure as for the bromo nucleosides **6** and **7**. However, transesterification reactions between the methyl ester compounds **10** and **11** and the phosphonyl derivative **5** gave rise to the formation of a complex mixture of reaction compounds.

LC/MS experiments proved that **12** and **13** were the main compounds. These could be isolated by CC. The differentiation between (**14**, **15**) and (**16**, **17**) could be made by tandem mass spectrometry. This complex reaction mixture can probably be avoided by the use of dimethyl (tosyloxymethyl)phosphonate instead of **5**. This could however not be checked because of a shortage of the ester derivatives **10** and **11**.



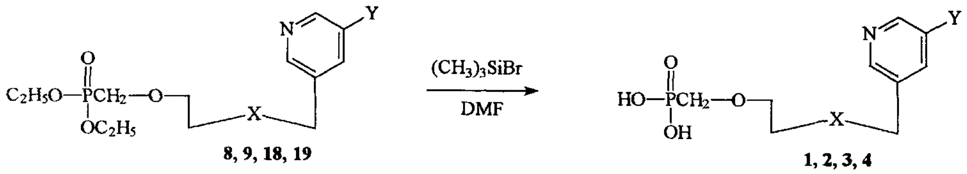
10, 12, 14, 16 : X=O
11, 13, 15, 17 : X=S

An ammonolysis reaction easily converted the ester derivatives **12** and **13** into the desired amide compounds **18** and **19**. The latter products could be purified by CCTLC and were obtained in 85-92% yield.



12, 18 : X=O
13, 19 : X=S

Treatment of the phosphonate ester derivatives with $(CH_3)_3SiBr$ (10 eq.) in dry DMF and stirring this mixture at room temperature for 3 days gave the aimed nucleoside phosphonates. These products could be purified by reversed phase (RP) HPLC-chromatography. The phosphonyl derivatives **1**, **2**, **3** and **4** were obtained in 45-60% yield.



8, 18, 1, 3 : X=O
9, 19, 2, 4 : X=S

8, 9, 1, 2 : Y=Br
18, 19, 3, 4 : Y=CONH2

TABLE 1.

Compound	IC$_{50}$ (μg/ml)					
	CMV (AD-169)	CMV (Davis)	VZV TK$^+$ (OKA)	VZV TK$^+$ (YS)	VZV TK$^-$ (07/1)	VZV TK$^-$ (YS/R)
4	>50	>50	43	36	50	33

BIOLOGICAL STUDIES.

All phosphonate nucleosides (**1** to **4**) were evaluated for their inhibitory effect on the replication of : parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures; vesicular stomatitis virus (VSV), Coxsackie virus B4 and respiratory syncytial virus in HeLa cell cultures; thymidine kinase-deficient (TK$^-$) strains of varicella-zoster virus (VZV) (07/1 and YS/R), wild-type VZV (OKA and YS) and cytomegalovirus (CMV) strains (AD-169 and Davis) in human embryonic lung (HEL) cells. Only compound **4** showed marginal antiviral activity at concentrations up to 400 μg/ml, as shown in TABLE 1. Neither anti-HIV-1, nor anti-HIV-2 activity was observed in human T-lymphocyte (CEM) cell cultures (EC$_{50}$>200 μg/ml).

The phosphonates **1** to **4** were also evaluated for their cytostatic activity against murine leukemia L1210 cells and human T-lymphocyte Molt4/clone 8 and CEM/0 cells. Compounds **1**, **3** and **4** showed cytostatic activity at concentrations up to 200 μg/ml. The corresponding nucleosides (**6**, **7**, **20** and **21**), as well as 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) are given for comparison (TABLE 2).

The minimum cytotoxic concentration (i.e. the compound concentration required to cause a microscopically detectable alteration of HeLa, HEL and Vero cell morphology) of all tested compounds was higher than 400 μg/ml.

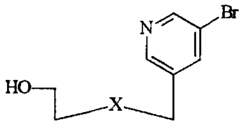
From these results we can conclude that the introduction of a phosphonomethyl group in our compounds slightly enhances the *in vitro* biological activity.

EXPERIMENTAL.

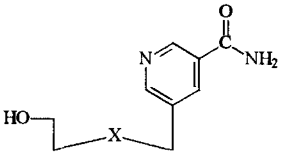
General methods.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz Varian Unity 400

TABLE 2.



6 : X=O
7 : X=S



20 : X=O
21 : X=S

	IC_{50}[a] (μg/ml)			
	L1210/0	Molt/4F	Molt4/C8	CEM
1	132	-	>200	113
2	>200	-	>200	>200
3	142	-	166	132
4	130	-	103	106
6	>200	>200	-	>200
7	>200	-	115±47	96±6.5
20	>200	>200	-	>200
21	>200	-	>200	>200
PMEA	1.8±0.16	-	13±1.1	24±18
PMEDAP	0.43±0.14	-	4.0±1.7-	3.8±0.6

a : 50 % inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

spectrometer. DCI-mass spectra were recorded 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 0.01 mmHg. The electrospray (ES) mass spectra were recorded on a VG Quattro II triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a Kontron HPLC-system consisting of a Model 325 pump, a Model 465 HPLC autosampler and a Model 332 HPLC detector set at 260 nm. For the LC/MS experiments an Econosphere 18 RP 5 (25cm x 4.6mm I.D.) column was used. The products were eluted using an isocratic run of 70%/30% MeOH/NH_4OAc (0.01 M) at a flow rate of 1 mL/min. The recordings were performed in the negative ion (ES-) or the positive ion (ES+) mode.

Post-column splitting was accomplished using a 1/20 Acurate™ splitter (LC Packings, Amsterdam, The Netherlands). The capillary voltage was c. +3 kV in the case of (+) ES and c. -3 kV in the case of (-) ES. The sample cone voltage was set at 40 V. The flow rate of the drying gas was 350 L/h and 20L/h for the nebulising gas.

Low-energy collision-activated decomposition (CAD) performed on the $[MH]^+$ fragments was obtained using Argon as a collision gas. The gas pressure was 1.6×10^{-3} mbar. The collision energy was 35 eV.

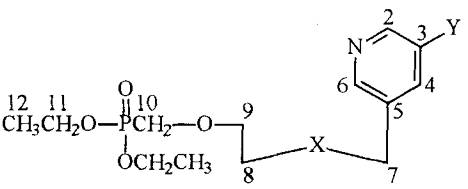
Analytical TLC was performed on silica plates (Kieselgel 60 F_{254} Merck, Darmstadt, 0.25 mm). Preparative centrifugal circular thin layer chromatography (CCTLC) was carried out on a Chromatotron® (Harrison Research, Palo Alto, CA). Stationary phase : Kieselgel 60 PF_{254} gipshaltig, Merck, layer thickness 2 mm, flow rate 5 ml/min. Column chromatography employed Merck silica gel (Kieselgel 60, 230-400 Mesh ASTM). HPLC analyses were carried out on a Varian Vista 5000 HPLC apparatus, equipped with an Econosphere 18 RP 5 (25 cm x 4.6 mm I.D.) column. For the preparative separations we used a SepTech Novaprep® 5000 apparatus equipped with an Econosphere 18 RP 10 (25cm x 22.5 mm I.D.) column.

DMF was dried by distillation from CaH_2 under reduced pressure. Diethylphosphite, paraformaldehyde, and bromotrimethylsilane were purchased from Acros Chimica (Beerse, Belgium).

Structure identification by NMR.

Structure identification was done by 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectroscopy. The spectra recorded in $CDCl_3$ or CD_3OD used TMS or the residual solvent signal [$CDCl_3$: $\delta = 77.0$ ppm (^{13}C) and 7.27 ppm (^{1}H); CD_3OD : $\delta =$ 49.0 ppm (^{13}C) and 3.31 ppm (^{1}H)] as internal reference. When NMR spectra were recorded in D_2O, dioxane was used as internal reference [$\delta = 66.3$ ppm (^{13}C) and 3.53 ppm (^{1}H)].

Basic numbering of our compounds was done as follows :



The carbon and hydrogen atoms of the nucleoside moiety were assigned mainly using results reported earlier by us.[13,14]

Synthesis.

3-Bromo-5-[[[2-(diethylphosphono)methoxy]ethoxy]methyl]pyridine (8) and 3-bromo-5-[[[2-(diethylphosphono)methoxy]ethylthio]methyl]-pyridine (9).

A three-necked flask (100 ml), equipped with a N_2-inlet adaptor, was charged with the bromo nucleoside (**6**, **7**) (ca. 2.5 mmole) and **5** (2 eq.) in dry DMF (30 ml) under N_2-atmosphere at 0°C. To this solution NaH (2 eq.) was added. This mixture was then stirred at room temperature for 3 h. After this period EtOAc (20 ml) and H_2O (20 ml) were added and the 2 layers were separated. The aqueous layer was extracted a few times more with EtOAc and the combined organic layers were washed with a saturated $NaHCO_3$-solution (30 ml), dried over anhydrous $MgSO_4$, filtered and concentrated. A first purification was done by column chromatography (CC) (15 cm x 4 cm I.D.) on silica gel, eluting with CH_2Cl_2/CH_3OH (95:5). Further purification was carried out using preparative CCTLC with the same eluent. This provided the coupling products **8** and **9** as a colourless oil.

Yields : (**8**) : 39%; (**9**) : 30%.

^{13}C-NMR.

(**8**) : ($CDCl_3$, TMS) : δ 150.2 (C-2), 147.1 (C-6), 137.7 (C-4), 135.7 (C-5), 120.8 (C-3), 72.7 (d, J=9.2 Hz, C-9), 70.2 (C-8), 70.0 (C-7), 65.8 (d, J=166.5 Hz, C-10), 62.4 (d, J=5.9 Hz, C-11), 16.5 (d, J=5.3 Hz, C-12).

(**9**) : ($CDCl_3$, solv. ref.) : δ 149.6 (C-2), 148.3 (C-6), 139.0 (C-4), 136.1 (C-5), 120.7 (C-3), 73.5 (d, J=10.7 Hz, C-9), 65.3 (d, J=166.3 Hz, C-10), 62.4 (d, J= 6.1 Hz, C-11), 33.2 (C-8), 30.6 (C-7), 16.5 (d, J=6.1 Hz, C-12).

^{1}H-NMR.

(**8**) : ($CDCl_3$, TMS) : δ 8.60 (1H, d, $^4J_{2,4}$=2.3 Hz, H-2), 8.48 (1H, d, $^4J_{4,6}$=1.7 Hz, H-6), 7.86 (1H, m, H-4), 4.57 (2H, $^4J_{4,7}$=0.5 Hz, H-7), 4.18 (4H, dq, $^3J_{11,12}$= 7.0 Hz, $J_{H\text{-}11,P}$=8.1 Hz, H-11), 3.87 (2H, d, $J_{H\text{-}10,P}$=8.1 Hz, H-10), 3.82 (2H, t, $^3J_{8,9}$=4.6 Hz, H-9), 3.70 (2H, t, $^3J_{8,9}$=4.6 Hz, H-8), 1.34 (6H, dt, $^3J_{11,12}$=7.0 Hz, $J_{H\text{-}12,P}$=0.3 Hz, H-12).

(**9**) : ($CDCl_3$, TMS) : δ 8.56 (1H, d, $^4J_{2,4}$=2.1 Hz, H-2), 8.48 (1H, d, $^4J_{4,6}$=1.8 Hz, H-6), 7.87 (1H, t, H-4), 4.19 (4H, dq, $^3J_{11,12}$=7.1 Hz, $J_{H\text{-}11,P}$=8.1 Hz, H-11), 3.80 (2H, d, $J_{H\text{-}10,P}$=8.4 Hz, H-10), 3.78 (2H, t, $^3J_{8,9}$=6.2 Hz, H-9), 3.77 (2H, s, H-7), 2.61 (2H, t, $^3J_{8,9}$=6.2 Hz, H-8), 1.35 (6H, t, $^3J_{11,12}$=7.1 Hz, H-12).

DCI-mass spectrometry (NH_3).

(**8**) : m/z = 382 ($[MH]^+$ (^{79}Br), 100%).

(**9**) : m/z = 398 ($[MH]^+$ (^{79}Br), 100%).

Elemental analysis for $C_{13}H_{21}Br_1N_1O_5P_1$ (**8**) : calcd. C, 40.94%; H, 5.55%; N, 3.68%. Found : C, 40.90%; H, 5.37%; N, 3.73%.

Elemental analysis for $C_{13}H_{21}Br_1N_1O_4P_1S_1$ (**9**) : calcd. C, 39.29%; H, 5.33%; N, 3.53%. Found : C, 39.21%; H, 5.28%; N, 3.70%.

3-Ethoxycarbonyl-5-[[[2-(diethylphosphono)methoxy]ethoxy]methyl]-pyridine (12) and 3-ethoxycarbonyl-5-[[[2-(diethylphosphono)-methoxy]ethylthio]methyl]pyridine (13).

The coupling reactions of the methyl ester nucleosides **10** and **11** with **5** were carried out analogously as described for the bromo derivatives. The complex mixtures were first purified by CC (30 cm x 4 cm I.D.) on silica gel, eluting with CH_2Cl_2/CH_3OH (95:5) and then by preparative CCTLC with the same eluent (**12** R_f = 0.55) or with CH_2Cl_2/CH_3OH (97:3) (**13**, R_f = 0.48). The coupled products **12** and **13** were obtained as a pale yellow oil.

Yields : (**12**) : 20%; (**13**) : 25%.

^{13}C-NMR.

(12) : ($CDCl_3$, solv. ref.) : δ 165.1 (C=O), 152.5 (C-6), 150.1 (C-2), 136.1 (C-4), 133.5 (C-5), 126.0 (C-3), 72.5 (d, J=10.0 Hz, C-9), 70.3 (C-8), 70.0 (C-7), 65.5 (d, J=166.3 Hz, C-10), 62.3 (d, J=3.9 Hz, C-11), 61.4 ($-COO\underline{C}H_2CH_3$), 16.4 (d, J=6.1 Hz, C-12), 14.2 ($-COOCH_2\underline{C}H_3$).

(13) : ($CDCl_3$, solv. ref.) : δ 165.1 (C=O), 153.6 (C-6), 149.5 (C-2), 137.2 (C-4), 134.2 (C-5), 126.3 (C-3), 73.2 (C-9), 65.3 (d, J=168.0 Hz, C-10), 62.4 (d, J= 6.0 Hz, C-11), 61.5 ($-COO\underline{C}H_2CH_3$), 33.5 (C-8), 30.7 (C-7), 16.5 (d, J=6.1 Hz, C-12), 14.3 ($-COOCH_2\underline{C}H_3$).

^{1}H-NMR.

(12) : ($CDCl_3$, TMS) : δ 9.15 (1H, d, $^4J_{2,4}$=2.0 Hz, H-2), 8.74 (1H, d, $^4J_{4,6}$=2.2 Hz, H-6), 8.27 (1H, t, H-4), 4.64 (2H, s, H-7), 4.42 (2H, q, ^3J=7.2 Hz, $-COOC\underline{H_2}CH_3$), 4.17 (4H, dq, $^3J_{11,12}$=7.1 Hz, $J_{H-11,P}$=7.8 Hz, H-11), 3.87 (2H, d, $J_{H-10,P}$=7.8 Hz, H-10), 3.83 (2H, t, $^3J_{8,9}$=4.7 Hz, H-9), 3.72 (2H, t, $^3J_{8,9}$=4.7 Hz, H-8), 1.42 (3H, t, ^3J=7.2 Hz, $-COOCH_2C\underline{H_3}$), 1.34 (6H, t, $^3J_{11,12}$=7.1 Hz, H-12).

(13) : ($CDCl_3$, TMS) : δ 9.10 (1H, d, $^4J_{2,4}$=2.0 Hz, H-2), 8.72 (1H, d, $^4J_{4,6}$=2.3 Hz, H-6), 8.28 (1H, t, H-4), 4.42 (2H, q, ^3J=7.2 Hz, $-COOC\underline{H_2}CH_3$), 4.18 (4H, dq, $^3J_{11,12}$=7.1 Hz, $J_{H-11,P}$=8.2 Hz, H-11), 3.85 (2H, s, H-7), 3.80 (2H, d, $J_{H-10,P}$= 8.2 Hz, H-10), 3.77 (2H, t, $^3J_{8,9}$=6.3 Hz, H-9), 2.63 (2H, t, $^3J_{8,9}$=6.3 Hz, H-8), 1.42 (3H, t, ^3J=7.2 Hz, $-COOCH_2C\underline{H_3}$), 1.35 (6H, dt, $^3J_{11,12}$=8.2 Hz, $J_{H-12,P}$= 0.5 Hz, H-12).

ES-mass spectrometry (ES+).

(12) : m/z = 376 ($[MH]^+$, 100%).

(13) : m/z = 392 ($[MH]^+$, 100%).

Elemental analysis for $C_{16}H_{26}N_1O_7P_1$ **(12)** : calcd. C, 51.18%; H, 6.98%; N, 3.73%. Found : C, 51.09%; H, 6.92%; N, 3.79%.

Elemental analysis for $C_{16}H_{26}N_1O_6P_1S_1$ **(13)** : calcd. C, 49.09%; H, 6.70%; N, 3.58%. Found : C, 49.14%; H, 6.79%; N, 3.51%.

3-Carbamoyl-5-[[[2-(diethylphosphono)methoxy]ethoxy]methyl]-pyridine (18) and 3-carbamoyl-5-[[[2-(diethylphosphono)methoxy]-ethylthio]methyl]pyridine (19).

A flask of 100 ml was charged with the ester derivative (**12** or **13**) (ca. 0.5 mmole), dissolved in 50 ml CH_3OH. This solution was cooled to 0°C and saturated with NH_3-gas. After being stirred for 3 days at room temperature, the reaction mixture was concentrated *in vacuo*. The products were first purified by CC (5 cm x 4 cm I.D.) on silica gel, eluting with CH_2Cl_2/CH_3OH (90:10), and further by preparative CCTLC with the same eluent. The amides **18** and **19** were obtained as a colourless oil.

Yields : (**18**) : 90%; (**19**) : 92%.

^{13}C-NMR.

(**18**) : ($CDCl_3$, solv. ref.) : δ 167.5 (C=O), 151.3 (C-6), 148.7 (C-2), 134.4 (C-4), 133.5 (C-5), 129.0 (C-3), 72.7 (d, J=11.4 Hz, C-9), 70.1 (C-8), 69.8 (C-7), 65.5 (d, J=167.9 Hz, C-10), 62.5 (d, J=6.8 Hz, C-11), 16.4 (d, J=5.3 Hz, C-12).

(**19**) : ($CDCl_3$, solv. ref.) : δ 167.4 (C=O), 153.2 (C-6), 148.3 (C-2), 135.7 (C-4), 133.9 (C-5), 128.3 (C-3), 75.2 (d, J=12.2 Hz, C-9), 65.3 (d, J=168.6 Hz, C-10), 62.7 (d, J=6.8 Hz, C-11), 33.4 (C-8), 29.7 (C-7), 16.5 (d, J=5.3 Hz, C-12).

^{1}H-NMR.

(**18**) : ($CDCl_3$, TMS) : δ 9.08 (1H, br, H-2), 8.65 (1H, br, H-6), 8.32 (1H, br, H-4), 7.63 and 6.13 (2H, br, -NH_2), 4.65 (2H, s, H-7), 4.15 (4H, dq, $^3J_{11,12}$= 7.2 Hz, $J_{H\text{-}11,P}$=7.5 Hz, H-11), 3.87 (2H, d, $J_{H\text{-}10,P}$=8.5 Hz, H-10), 3.81 (2H, t, $^3J_{8,9}$=4.7 Hz, H-9), 3.72 (2H, t, $^3J_{8,9}$=4.7 Hz, H-8), 1.32 (6H, t, $^3J_{11,12}$=7.2 Hz, H-12).

(**19**) : ($CDCl_3$, TMS) : δ 9.08 (1H, d, $^4J_{2,4}$=1.8 Hz, H-2), 8.75 (1H, d, $^4J_{4,6}$=1.5 Hz, H-6), 8.29 (1H, t, H-4), 7.71 and 5.67 (2H, br, -NH_2), 4.16 (4H, dq, $^3J_{11,12}$= 7.1 Hz, $J_{H\text{-}11,P}$=7.5 Hz, H-11), 3.90 (2H, s, H-7), 3.83 (2H, t, $^3J_{8,9}$=7.1 Hz, H-9), 3.79 (2H, d, $J_{H\text{-}10,P}$=8.9 Hz, H-10), 2.54 (2H, t, $^3J_{8,9}$=7.1 Hz, H-8), 1.34 (6H, t, $^3J_{11,12}$=7.1 Hz, H-12).

DCI-mass spectrometry (NH_3).

(**18**) : m/z = 347 ($[MH]^+$, 100%).

(**19**) : m/z = 363 ($[MH]^+$, 100%).

Elemental analysis for $C_{14}H_{23}N_2O_6P_1$ (**18**) : calcd. C, 48.54%; H, 6.70%; N, 8.09%. Found : C, 48.43%; H, 6.59%; N, 8.21%.

Elemental analysis for $C_{14}H_{23}N_2O_5P_1S_1$ (**19**) : calcd. C, 46.40%; H, 6.40%; N, 7.73%. Found : C, 46.35%; H, 6.48%; N, 7.65%.

3-Bromo-5-[[2-(phosphonomethoxy)ethoxy]methyl]pyridine (1), 3-bromo-5-[[2-(phosphonomethoxy)ethylthio]methyl]pyridine (2), 3-carbamoyl-5-[[2-(phosphonomethoxy)ethoxy]methyl]pyridine (3) and 3-carbamoyl-5-[[2-(phosphonomethoxy)ethylthio]methyl]pyridine (4).

A solution of the diethyl phosphonyl derivative (**8**, **9**, **18** or **19**) (ca. 0.3 mmole) in dry DMF (5 ml) at room temperature under N_2-atmosphere was treated with bromotrimethylsilane (10 eq.) and the mixture was stirred for 24 hours. After this period an additional portion of bromotrimethylsilane (5 eq.) was added and the mixture was stirred for 2 more days and concentrated *in vacuo*. The residue was evaporated from H_2O (2 x 5 ml). The phosphonyl derivatives **1** to **4** were purified by RP HPLC-chromatography on an Econosphere 18RP10 column (25 cm x 22.5 mm I.D.), eluting with H_2O/CH_3OH (85:15) (**1**, **2**) or H_2O 100%) (**3**, **4**). This afforded the nucleoside phosphonates **1** to **4** as white solids.

Yields : (**1**) : 54%; (**2**) : 45%; (**3**) : 58%; (**4**) : 59%.

^{13}C-NMR.

(**1**) : (CD_3OD, solv. ref.) : δ 150.4 (C-2), 147.9 (C-6), 139.8 (C-4), 138.6 (C-5), 122.1 (C-3), 73.4 (d, J=8.3 Hz, C-9), 71.2 (C-8), 70.6 (C-7), 69.1 (d, J=166.3 Hz, C-10).

(**2**) : (CD_3OD, solv. ref.) : δ 149.7 (C-2), 149.2 (C-6), 141.0 (C-4), 139.2 (C-5), 121.8 (C-3), 74.1 (d, J=11.4 Hz, C-9), 69.2 (d, J=158.7 Hz, C-10), 33.6 (C-8), 31.6 (C-7).

(**3**) : (D_2O, dioxane ref.) : δ 170.0 (C=O), 151.1 (C-6), 147.0 (C-2), 136.0 (C-4), 133.8 (C-5), 129.2 (C-3), 71.3 (d, J=10.7 Hz, C-9), 69.3 (C-8), 69.1 (C-7), 66.9 (d, J=156.4 Hz, C-10).

(4) : (D_2O, dioxane ref.) : δ 169.9 (C=O), 151.6 (C-6), 146.1 (C-2), 136.4 (C-4), 135.2 (C-5), 129.1 (C-3), 71.2 (d, J=10.7 Hz, C-9), 66.5 (d, J=156.4 Hz, C-10), 31.9 (C-8), 29.8 (C-7).

^{1}H-NMR.

(1) : (CD_3OD, solv. ref.) : δ 8.56 (1H, br, H-2), 8.51 (1H, br, H-6), 8.04 (1H, br, H-4), 4.63 (2H, s, H-7), 3.77 (2H, br, H-9), 3.73 (2H, br, H-8), 3.63 (2H, d, $J_{H\text{-}10,P}$=8.8 Hz).

(2) : (D_2O, dioxane ref.) : δ 8.33 (1H, br, H-2), 8.26 (1H, br, H-6), 7.89 (1H, s, H-4), 3.63 (2H, s, H-7), 3.50 (2H, t, $^3J_{8,9}$=6.3 Hz, H-9), 3.35 (2H, d, $J_{H\text{-}10,P}$= 8.7 Hz, H-10), 2.49 (2H, t, $^3J_{8,9}$=6.3 Hz, H-8).

(3) : (D_2O, dioxane ref.) : δ 8.70 (1H, br, H-2), 8.53 (1H, br, H-6), 8.03 (1H, s, H-4), 4.50 (2H, s, H-7), 3.57 (4H, br, H-8 and H-9), 3.45 (2H, d, $J_{H\text{-}10,P}$=8.4 Hz, H-10).

(4) : (D_2O, dioxane ref.) : δ 8.62 (1H, br, H-2), 8.50 (1H, br, H-6), 8.02 (1H, s, H-4), 4.51 (2H, s, H-7), 3.51 (2H, t, $^3J_{8,9}$=6.2 Hz, H-9), 3.40 (2H, d, $J_{H\text{-}10,P}$= 8.6 Hz, H-10), 2.48 (2H, t, $^3J_{8,9}$=6.3 Hz, H-8).

ES-mass spectrometry (ES-).

(1) : m/z = 324 ($[M\text{-}H]^-$, ^{79}Br, 100%).

(2) : m/z = 340 ($[M\text{-}H]^-$, ^{79}Br, 100%).

(3) : m/z = 289 ($[M\text{-}H]^-$, 100%).

(4) : m/z = 305 ($[M\text{-}H]^-$, 100%).

Elemental analysis for $C_9H_{13}Br_1N_1O_5P_1$ (1) : calcd. C, 33.23%; H, 4.03%; N, 4.31%. Found : C, 33.30%; H, 3.94%; N, 4.37%.

Elemental analysis for $C_9H_{13}Br_1N_1O_4P_1S_1$ (2) : calcd. C, 31.68%; H, 3.84%, N, 4.11%. Found : C, 31.61%, H, 3.80%; N, 4.18%.

Elemental analysis for $C_{10}H_{15}N_2O_6P_1$ (3) : calcd. C, 41.37%; H, 5.21%; N, 9.66%. Found : C, 41.45%; H, 5.13%; N, 9.61%.

Elemental analysis for $C_{10}H_{15}N_2O_5P_1S_1$ (4) : calcd. C, 39.21%; H, 4.94%, N, 9.15%. Found : C, 39.08%; H, 4.87%; N, 9.28%.

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