SYNTHESIS AND ANTIVIRAL ACTIVITY OF ACYCLIC NUCLEOTIDE ANALOGUES DERIVED FROM 6-(AMINOMETHYL)PURINES AND PURINE-6-CARBOXAMIDINES

Michal HOCEK[a1], Milena MASOJIDKOVA[a], Antonin HOLY[a], Graciela ANDREI[b], Robert SNOECK[b], Jan BALZARINI[b] and Erik DE CLERCQ[b]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] hocek@uochb.cas.cz*

[b] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

Received May 31, 1996
Accepted July 9, 1996

Dedicated to the memory of Dr Zdenek Arnold.

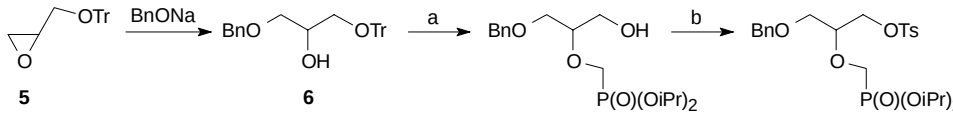
The synthesis of a series of 9-(2-phosphonomethoxyalkyl) derivatives of 6-(aminomethyl)purine **11**, 2-amino-6-(aminomethyl)purine **12** and purine-6-carboxamidine **14** is reported. The 6-cyanopurines **1** and **2** were selectively alkylated with 2-[bis(isopropyloxy)phosphonylmethoxy]alkyl synthons **3** and **4** at the 9-position. Catalytic hydrogenation of the obtained 9-{2-[bis(isopropyloxy)phosphonylmethoxy]-alkyl}-6-cyanopurines **9** and **10** followed by treatment with bromotrimethylsilane afforded the title compounds **11** and **12**. Analogous acyclic nucleotides derived from purine-6-carboxamidines **14** were prepared from the cyanopurines **9a** and **10a** by treatment with sodium methoxide and ammonium chloride followed by deprotection. Compounds **11** and **12** exhibited moderate activity (MIC_{50} = 3–50 μg/ml) against herpes simplex virus type 1, varicella-zoster virus and Moloney murine sarcoma virus in vitro.
Key words: Acyclic nucleoside phosphonates; Phosphonomethoxyalkylpurine derivatives; 6-Cyanopurines; 6-(Aminomethyl)purines; Purine-6-carboxamidines; Antivirals.

In the structure–activity relationship study of a series of antiviral phosphonomethoxyalkyl derivatives of pyrimidine and purine bases (for reviews see ref.[1]) it was found that the presence of an amino group at the heterocyclic moiety is necessary for the antiviral activity. Recently, several *N*(6)-monosubstituted and *N*(6)-disubstituted derivatives of adenine and 2,6-diaminopurine were also found to be active[2].

To study the role of the amino group at the purine base in the biological activity of these compounds a series of acyclic nucleotide analogues bearing the strongly basic 2-(aminomethyl) function on the purine ring was prepared[3]. All compounds of that series were found to be inactive. As a continuation of this study we describe here the synthesis of 9-[2-(phosphonomethoxy)alkyl] derivatives of 6-(aminomethyl)purines and purine-6-carboxamidines. The 6-(aminomethyl)purines are reported[4] to be unstable. They can be prepared[4,5] by catalytic hydrogenation of 6-cyanopurines and were usually

isolated[5] as stable *N*-acyl derivatives. On the contrary, the afore-mentioned phosphonomethoxyalkyl derivatives of 2-(aminomethyl)purines are stable, probably due to zwitterion formation.

A facile method of preparation of 6-cyanopurines **1** and **2** was recently reported[6]. Synthons **3** and **4** were used for the introduction of the phosphonomethoxyalkyl group. While the 2-phosphonomethoxyethyl (PME) synthon **3** was already known[7], the racemic 3-hydroxy-2-phosphonomethoxypropyl (HPMP) synthon **4** containing a hydrogenolytically cleavable *O*-benzyl protecting function[8] was prepared from tritylglycidol **5**. Sodium benzoxide was used for oxirane ring opening of the glycidol **5** that proceeded regioselectively to afford 3-*O*-benzyl-1-*O*-tritylglycerol **6**. The 2-hydroxy group was alkylated with diisopropyl tosyloxymethylphosphonate[9] **7** and the trityl function was cleaved[10] using wet Dowex 50 (H^+) in methanol to give the protected 2-*O*-phosphonomethylglycerol **8**. The target HPMP-synthon **4** was prepared by tosylation of this intermediate with tosyl chloride in pyridine (Scheme 1).



5 **6** **8** **4**

a) 1. $TsOCH_2P(O)(OiPr)_2$ (**7**), NaH, 2. Dowex 50 (H^+); b) TsCl, Py

SCHEME 1

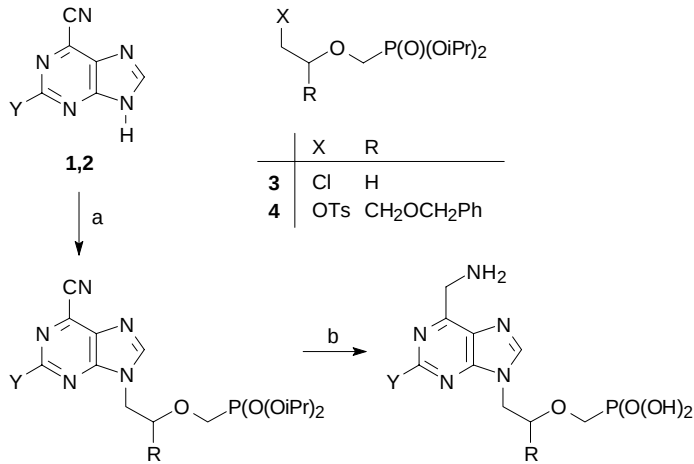
The alkylation of 6-cyanopurines **1** and **2** with the synthons **3** and **4** under standard conditions using sodium hydride in dimethylformamide at 100 °C gave only traces of the alkylated products. With cesium carbonate as a base the 9-substituted 6-cyanopurine phosphonates **9** and **10** were obtained selectively with only trace impurities of other regioisomers. Preparative TLC chromatography afforded pure oily phosphonates **9** and **10** in the yields of 48–60% that were used in further steps (Scheme 2).

The protected 6-cyanopurines **9** or **10** on treatment with bromotrimethylsilane (TMSBr) gave a complicated pattern of reaction products. Therefore, the compounds **9** and **10** were first hydrogenated. After two days the TLC detection showed that the cyano group was completely reduced. In the case of the *O*-benzyl protected compounds **9b** and **10b**, however, the removal of the benzyl group was not quantitative. The sluggish reaction could be explained by a catalyst poisoning by the aminomethyl group; a similar effect was recently described[11] for selective inhibition of hydrogenolytic cleavage of the *O*-benzyl function in the presence of ammonia.

Without isolation or characterization, the crude products were treated immediately with TMSBr to cleave the isopropyl ester groups. This reaction was not quantitative either and, due to lower solubility of the intermediates in acetonitrile, in some cases

dimethylformamide had to be used as a solvent. Standard purification by a combination of cation and anion exchange chromatography did not afford pure products; in the case of compounds **11b**, **12a** and **12b** preparative reversed phase HPLC was used to remove the phosphonate monoester and the *O*-benzyl protected side-products. In one case, the pure *O*-benzyl byproduct **12c** was isolated and characterized (Scheme 2).

The 9-substituted 6-cyanopurines **9a** and **10a** were converted to the 6-carboxamidino derivatives **14** with the use of the recently published method[12] for the preparation of hetarylcarboxamidines. On treatment with a catalytic amount of sodium methoxide in methanol the compounds **9a** and **10a** formed imidate intermediates that were without isolation treated with ammonium chloride to afford the protected amidine derivatives **13**. While the reaction of compound **9a** with methanol proceeded at room temperature, the reaction of the compound **10a** had to be performed at reflux temperature to reach com-

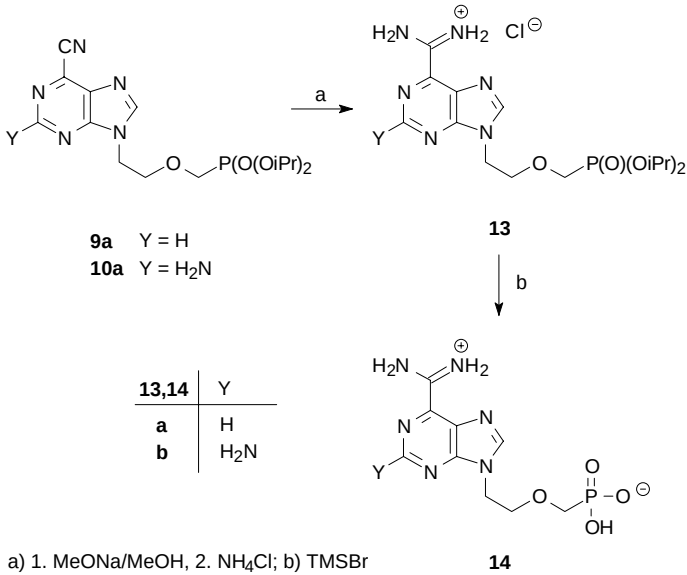


	Y	R
1	H	
2	H_2N	
9a	H	H
9b	H	CH_2OBn
10a	H_2N	H
10b	H_2N	CH_2OBn
11a	H	H
11b	H	CH_2OH
12a	H_2N	H
12b	H_2N	CH_2OH
12c	H_2N	CH_2OBn

a) **3** or **4**, Cs_2CO_3, DMF;
b) 1. H_2, Pd-C; 2. TMSBr, CH_3CN

SCHEME 2

plete conversion. The amidines **13** were without isolation deprotected using TMSBr in dimethylformamide to afford the desired 9-(2-phosphonomethoxyethyl) derivatives of purine-6-carboxamidine **14a** and of 2-aminopurine-6-carboxamidine **14b** in the yields of 44% and 51%, respectively, that were easily isolated by ion exchange chromatography (Scheme 3).



SCHEME 3

Although the method does not offer high yields and the purification of products is quite complicated, the acyclic nucleotide analogues derived from 6-(aminomethyl)purines **11a**, **11b** and **12a**, **12b** were prepared in amounts and purity sufficient for the biological activity tests. The compounds were much less stable than the analogous phosphonate derivatives of 2-(aminomethyl)purines[3] and were characterized as hygroscopic lyophilizates that quickly turned violet (compounds **11**) or green (compounds **12**) on exposure to air. The amidine compounds **14**, on the other hand, were stable.

Similarly to the 2-(aminomethyl)purine analogues[3], the electrophoretical mobilities of compounds **11**, **12** and **14** are substantially lower than those of adenine derivatives. UV spectra of the compounds **11** exhibited maxima at 265 nm, while the fluorescent compounds **12** displayed maxima at 306 nm with significant bathochromic shift to 317 nm in an acidic medium. The UV spectrum of the compound **14a** exhibited a maximum at 296 nm with hypsochromic shift to 279 nm in an alkaline medium and compound **14b** absorbed at 361 nm with hypsochromic shift to 334 nm in alkalies. The alkali-induced

hypsochromic shifts of the amidine compounds indicate that the amidine function is fully protonated even at pH 7.

Structure of the compounds was proved by ^{1}H and ^{13}C NMR spectra. Chemical shifts of the base carbon atoms (Table I) are in good accord with the reported values for 6-mono- and 2,6-disubstituted purines[13] considering the shielding of the substituents CN, CH_2NH_2, $C(=NH)NH_2$ and NH_2. While the C-4 and C-5 signals of the free bases **1** and **2** are broad, the corresponding signals of the 9-substituted derivatives **9–14** are sharp due to the excluded 7H–9H tautomerism.

The acyclic nucleotide analogues **11**, **12** and **14** were tested in vitro for cytostatic activity (L-929, L-1210 and HeLa cells) and for inhibitory effect on the DNA viruses: herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), cytomegalovirus (CMV), varicella-zoster virus (VZV) and vaccinia virus, and retroviruses: Moloney murine sarcoma virus (MSV) and human immunodeficiency virus type 1 (HIV-1/IIIb) and type 2 (HIV-2/ROD). Compound **11a** exhibited a moderate inhibitory effect (MIC_{50} = 20 μg/ml) against HSV-1 (F); compounds **11b** and **12a** were active against both the TK^+ and TK^- VZV strains at concentrations varying between 3 and 33 μg/ml and against MSV at 30 and 18 μg/ml, respectively. Also compound **12b** showed a moderate antiviral activity against VZV (MIC_{50} = 30–48 μg/ml). Other antiviral activity assays as well as cytostatic activity tests were negative. None of these compounds showed any considerable cell toxicity.

In conclusion, the replacement of the 6-amino or (di)alkylamino group of the strongly active purine nucleotide analogues by the aminomethyl function (derivatives **11** and **12**) caused substantial decrease of activity in most antiviral assays but still some activity against HSV-1, VZV and MSV was preserved. Replacement of the amino group by the carboxamidine function (derivatives **14**) led to complete loss of antiviral activity.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 60 °C/2 kPa over P_2O_5. Melting points were determined on a Kofler block and are uncorrected. TLC was performed on Silufol UV_{254} plates (Kavalier Votice, Czech Republic) (A) in $CHCl_3$–MeOH (80 : 20) mixture or (B) in ethyl acetate. Preparative TLC was carried out on 40 × 17 × 0.4 cm plates of silica gel containing a UV indicator. Reversed phase preparative HPLC was carried out on a Separon SGX column (300 × 16 mm) in water for 20 min, followed by a linear gradient of methanol (0–10% in 30 min). Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate at pH 7.5 and the electrophoretical mobilities are referenced to uridine 3′-phosphate. NMR spectra were measured on a Varian Unity 500 spectrometer (500 MHz for ^{1}H and 125.7 MHz for ^{13}C NMR) in hexadeuteriodimethyl sulfoxide referenced to the solvent signals 2.5 ppm for ^{1}H and 39.7 ppm for ^{13}C NMR, or in deuterium oxide containing sodium deuteroxide with sodium disilapentanesulfonate as internal standard for ^{1}H and dioxane as external standard for ^{13}C NMR (δ(dioxane) = 66.86). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB technique (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). UV

TABLE I
^{13}C NMR spectra (δ, ppm) of compounds **1**, **2**, **9–14**[a,b]

Compound	C-2	C-4	C-5	C-6	C-8	C-1″	C-1′	C-2′[c]	C-3′	CH_2P[d]
1	152.11	156.04	134.14	127.57	150.64	114.95[c]	–	–	–	–
2	160.77	156.67	129.09	128.09	145.15	114.95[e]	–	–	–	–
9a	152.36	153.26	134.92	128.73	151.07	114.46[e]	43.43	69.81	–	64.64
9b	152.37	153.51	134.90	128.64	151.20	114.55[e]	44.54	77.77	68.80	63.59
10a	160.65	155.39	129.64	128.44	146.89	114.72[c]	42.64	69.89	–	64.72
10b	160.66	155.60	129.56	128.37	147.09	114.74[e]	43.88	77.85	68.86	63.65
11a	152.76	152.01	131.68	152.36	149.16	40.25[f]	44.77	71.26	–	68.16
11b	151.45	150.79	130.14	151.04	148.03	38.81[f]	43.98	79.43	59.82	65.97
12a	159.47	152.59	123.78	152.13	144.56	38.52[f]	42.68	69.92	–	66.74
12b	159.54	152.83	123.68	152.19	144.84	38.55[f]	43.31	79.60	59.95	65.94
12c	159.40	152.76	123.49	151.88	144.70	38.45[f]	43.36	77.46	67.94	65.72
13a	151.77	154.10	131.45	139.45	150.95	160.54[g]	43.60	69.89	–	64.66
13b	160.10	156.10	124.81	140.36	146.87	160.77[g]	42.82	69.90	–	64.73
14a	151.17	153.58	131.27	138.68	150.80	159.58[g]	43.66	69.67	–	66.77
14b	158.67	155.17	124.12	139.39	146.89	158.90[g]	43.25	68.77	–	68.52

[a] $(CD_3)_2SO$; spectra of compounds **11**, **12** and **14a** in D_2O; compound **14b** in D_2O + NaOD. [b] Spectra of compounds **9**, **10** and **13** contained additional signals of protecting group carbon atoms: 23.6–23.8 (CH_3); 70.24–70.33 (CH); spectra of compounds **9b**, **10b** and **12c** also: 72.66–72.81 (CH_2Ph); 127.72–138.20 (four aromatic carbon atoms). [c] Doublets, $^3J(P,C)$ = 10.7–12.7. [d] Doublets, $^1J(P,C)$ = 156–164. [e] C-1" = CN. [f] C-1" = CH_2NH_2. [g] C-1" = $C(NH)NH_2$.

absorption spectra (λ_{max}, nm; (ε)) were measured on a Beckman DU-65 spectrometer in aqueous solutions.

Dimethylformamide was distilled from P_2O_5 and stored over molecular sieves. Acetonitrile was refluxed with calcium hydride and distilled. The 6-cyanopurines[6] **1** and **2** as well as the phosphonate synthon[7] **3** were prepared according to the previously published methods.

1-Benzyloxy-3-(triphenylmethoxy)-2-propanol (**6**)

To a stirred solution of NaH (60% dispersion in mineral oil; 10.8 g, 289 mmol) in DMF (100 ml) a solution of benzyl alcohol (33.4 ml, 323 mmol) in DMF (50 ml) was added and the stirring at room temperature was continued for 15 min. Then a solution of trityloxymethyloxirane[14] (**5**; 17 g, 54 mmol) in DMF (50 ml) was added dropwise and the mixture was stirred at 100 °C for 2.5 h. After cooling, the mixture was diluted with water (150 ml) and extracted with ether (3 × 250 ml). The combined organic layers were dried with $MgSO_4$ and evaporated. Excess of benzyl alcohol was distilled off (bath temperature 100 °C, 130 Pa) and the residue was purified by column chromatography on silica gel (200 g, ethyl acetate–light petroleum 1 : 2) to afford compound **6** as an amorphous solid, m.p. 71–73 °C. Yield 19 g (83.3%). For $C_{29}H_{28}O_3$ (424.5) calculated : 82.05% C, 6.65% H; found: 81.57% C, 6.61% H. Mass spectrum (FAB), *m/z* (rel.%): 243 (100) [Tr], 165 (22) [M – Tr], 91 (38). ^{1}H NMR spectrum ($CDCl_3$): 3.20 dd, 1 H, *J*(1b,2) = 5.5, *J*(gem) = 9.5 (Hb-1); 3.26 dd, 1 H, *J*(1a,2) = 5.5, *J*(gem) = 9.5 (Ha-1); 3.56 dd, 1 H, *J*(3b,2) = 6.1, *J*(gem) = 9.5 (Hb-3); 3.62 dd, 1 H, *J*(3a,2) = 4.6, *J*(gem) = 9.5 (Ha-3); 4.00 m, 1 H (H-2); 4.54 s, 2 H (CH_2Ph); 7.20–7.35 m, 14 H (H-arom); 7.40–7.50 m, 6 H (H-arom).

Diisopropyl {1-[(Benzyloxy)methyl]-2-hydroxyethoxy}methylphosphonate (**8**)

To a stirred suspension of NaH (60% dispersion; 2.0 g, 50 mmol) in dry THF (50 ml) a solution of **6** (19 g, 44.8 mmol) in THF (50 ml) was slowly added and the suspension was stirred at room temperature for 1 h. Then a solution of diisopropyl tosyloxymethylphosphonate[9] (**7**; 18.2 g, 52 mmol) in THF (20 ml) was added dropwise and the stirring at room temperature was continued for 36 h. Methanol (50 ml) followed by ethyl acetate (250 ml) was added and the solution was washed with water (3 × 100 ml). The combined organic phases were dried over $MgSO_4$ and evaporated. The residue was refluxed with Dowex 50 (H^+) (40 ml) in methanol (240 ml) and water (60 ml) for 3 h, filtered, the Dowex was washed with methanol (100 ml), and the combined filtrates were evaporated. The residue was dissolved in ethyl acetate (350 ml) and washed with water (3 × 200 ml). The organic layer was dried over $MgSO_4$ and evaporated. Column chromatography of the residue (150 g silica gel, toluene–ethyl acetate gradient 0–100%) afforded compound **8** as a yellowish oil, R_F (B) 0.17. Yield 7.68 g (48%). Mass spectrum (FAB), *m/z* (rel.%): 361 (55) [M + H], 91 (100). ^{1}H NMR spectrum ($(CD_3)_2SO$): 1.21–1.24 m, 12 H (CH_3); 3.46–3.63 m, 5 H (H-1, H-2, H-3); 3.86 dd, 1 H, *J*(P,CHb) = 8.8, *J*(gem) = 13.9 (PC-Hb); 3.90 dd, 1 H, *J*(P,CHa) = 8.8, *J*(gem) = 13.9 (PC-Ha); 4.48 and 4.51 2 × d, 2 × 1 H, *J*(gem) = 12.2 (CH_2Ph); 4.59 m, 2 H (POCH); 4.69 t, 1 H, *J*(OH,CH_2) = 5.4 (OH); 7.30–7.40 m, 5 H (H-arom).

Diisopropyl {1-[(Benzyloxy)methyl]-2-(*p*-toluenesulfonyloxy)ethoxy}methylphosphonate (**4**)

To a stirred solution of **8** (7.52 g, 20.9 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (200 mg) in pyridine (70 ml) TsCl (4.58 g, 24 mmol) was added portionswise at –20 °C. The stirring was continued for 30 min at –20 °C and for 16 h at room temperature. Water (25 ml) was added and the mixture was concentrated in vacuo to about 50 ml and codistilled with water (20 ml). Ethyl acetate (250 ml) was added and the solution was washed successively with 0.1 M HCl (2 × 200 ml), 10% $NaHCO_3$

(2 × 200 ml) and water (8 × 200 ml). The organic layer was dried with $MgSO_4$ and evaporated to give compound **4** as yellowish oil, R_F (B) 0.49. Yield 8.6 g (80%). For $C_{24}H_{35}O_8PS$ (514.6) calculated: 56.02% C, 6.86% H, 6.02% P, 6.23% S; found: 55.57% C, 6.98% H, 6.29% P, 6.58% S. Mass spectrum (FAB), *m/z* (rel.%): 515 (55) [M + H], 431 (100). ^{1}H NMR spectrum ($(CD_3)_2SO$): 1.19–1.22 4 × d, 4 × 3 H, $J(CH_3,CH)$ = 6.1 (CH_3); 2.40 s, 3 H (CH_3-Ts); 3.46 dd, 1 H, J(1b,2) = 5.1, J(gem) = 10.5 (Hb-1); 3.50 dd, 1 H, J(1a,2) = 5.1, J(gem) = 10.5 (Ha-1); 3.77 dd, 1 H, J(P,CHb) = 8.8, J(gem) = 13.7 (PC-Hb); 3.83 dd, 1 H, J(P,CHa) = 9.0, J(gem) = 13.7 (PC-Ha); 3.84 m, 1 H (H-2); 4.03 dd, 1 H, J(3b,2) = 5.4, J(gem) = 10.5 (Hb-3); 4.17 dd, 1 H, J(3a,2) = 3.4, J(gem) = 10.5 (Ha-3); 4.42 s, 2 H (CH_2Ph); 4.56 dsept, 2 H, $J(CH,CH_3)$ = 6.1, J(P,OCH) = 7.8 (POCH); 7.22–7.35 m, 5 H (H-Ph); 7.79 d, 2 H and 7.46 d, 2 H, J = 8.3 (CH-Ts).

Alkylation of 6-Cyanopurines **1** and **2**. General Procedure

Cesium carbonate (490 mg, 1.5 mmol) was added to a solution of the 6-cyanopurine **1** or **2** (3 mmol) in DMF (30 ml) and the mixture was stirred at 100 °C for 1 h, then cooled to 50 °C and a solution of the synthon **3** or **4** (3.2 mmol) in DMF (5 ml) was added dropwise. The mixture was then stirred at 120 °C for 16 h (TLC detection showed that most of the starting material had disappeared). The solvent was evaporated, water (30 ml) was added and the mixture was extracted with ethyl acetate (3 × 50 ml). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was chromatographed on a preparative layer of silica gel. The obtained chromatographically homogeneous (TLC) oily compounds **9** and **10** were used in further steps.

9-{2-[Bis(isopropyloxy)phosphonylmethoxy]ethyl}-6-cyanopurine (**9a**), yield 48%, R_F (A) 0.50. For $C_{15}H_{22}N_5O_4P$. 1/2 H_2O (376.4) calculated: 47.86% C, 6.16% H, 18.61% N, 8.23% P; found: 47.98% C, 6.15% H, 18.55% N, 8.15% P. Mass spectrum (FAB), *m/z* (rel.%): 368 (55) [M + H]. ^{1}H NMR spectrum ($(CD_3)_2SO$): 1.08 d, 6 H and 1.14 d, 6 H, $J(CH_3,CH)$ = 6.1 (4 × CH_3); 3.78 d, 2 H, $J(P,CH_2)$ = 8.1 (PCH_2); 3.96 t, 2 H, J(2′,1′) = 5.1 (H-2′); 4.44 dsept, 2 H, $J(CH,CH_3)$ = 6.1, J(P,OCH) = 7.8 (2 × POCH); 4.54 t, 2 H, J(1′,2′) = 5.1 (H-1′); 8.89 s, 1 H (H-8); 9.14 s, 1 H (H-2).

9-{3-Benzyloxy-2-[bis(isopropyloxy)phosphonylmethoxy]propyl}-6-cyanopurine (**9b**), yield 53%, R_F (A) 0.73. Exact mass (EI HRMS) for $C_{23}H_{30}N_5O_5P$ calculated: 487.1985; found: 487.1918. Mass spectrum (FAB), *m/z* (rel.%): 488 (10) [M + H], 91 (100). ^{1}H NMR spectrum ($(CD_3)_2SO$): 1.06 d, 3 H, 1.115 d, 3 H, 1.12 d, 3 H and 1.16 d, 3 H, $J(CH_3,CH)$ = 6.1 (4 × CH_3); 3.58 dd, 1 H, J(3′b,2′) = 4.6, J(gem) = 10.7 (Hb-3′); 3.62 dd, 1 H, J(3′a,2′) = 4.6, J(gem) = 10.7 (Ha-3′); 3.78 dd, 1 H, J(P,CHb) = 9.3, J(gem) = 13.9 (PC-Hb); 3.89 dd, 1 H, J(P,CHa) = 8.8, J(gem) = 13.9 (PC-Ha); 4.11 m, 1 H (H-2′); 4.42 dsept, 1 H and 4.47 dsept, 1 H, $J(CH,CH_3)$ = 6.1, J(P,OCH) = 7.6 (2 × POCH); 4.47 d, 1 H and 4.49 d, 1 H, J(gem) = 12.0 (CH_2Ph); 4.51 dd, 1 H, J(1′b,2′) = 7.3, J(gem) = 14.6 (Hb-1′); 4.57 dd, 1 H, J(1′a,2′) = 3.9, J(gem) = 14.6 (Ha-1′); 7.30–7.40 m, 5 H (H-arom.); 8.83 s, 1 H (H-8); 9.12 s, 1 H (H-2).

2-Amino-9-{2-[bis(isopropyloxy)phosphonylmethoxy]ethyl}-6-cyanopurine (**10a**), yield 60%, R_F (A) 0.35. Exact mass (EI HRMS) for $C_{15}H_{23}N_6O_4P$ calculated: 382.1518; found: 382.1528. Mass spectrum (FAB), *m/z* (rel.%): 383 (40) [M + H], 299 (100). ^{1}H NMR spectrum ($(CD_3)_2SO$): 1.11 d, 6 H and 1.16 d, 6 H, $J(CH_3,CH)$ = 6.1 (4 × CH_3); 3.77 d, 2 H, $J(P,CH_2)$ = 8.3 (PCH_2); 3.88 t, 2 H, J(2′,1′) = 5.1 (H-2′); 4.26 t, 2 H, J(1′,2′) = 5.1 (H-1′); 4.47 dsept, 2 H, $J(CH,CH_3)$ = 6.1, J(P,OCH) = 7.6 (2 × POCH); 7.08 bs, 2 H (NH_2); 8.29 s, 1 H (H-8).

2-Amino-9-{3-benzyloxy-2-[bis(isopropyloxy)phosphonylmethoxy]propyl}-6-cyanopurine (**10b**), yield 54%, R_F (A) 0.65. Exact mass (EI HRMS) for $C_{23}H_{31}N_6O_5P$ calculated: 502.2094; found: 502.2048. Mass spectrum (FAB), *m/z* (rel.%): 503 (10) [M + H], 91 (100). ^{1}H NMR spectrum ($(CD_3)_2SO$): 1.08 d, 3 H; 1.13 d, 6 H; 1.17 d, 3 H, $J(CH_3,CH)$ = 6.1 (4 × CH_3); 3.54 dd, 1 H, J(3′b,2′) = 4.6, J(gem) = 10.5 (Hb-3′); 3.60 dd, 1 H, J(3′a,2′) = 4.6, J(gem) = 10.5 (Ha-3′); 3.74 dd, 1 H, J(P,CHb) = 9.3,

J(gem) = 13.9 (PC-Hb); 3.87 dd, 1 H, *J*(P,CHa) = 8.8, *J*(gem) = 13.9 (PC-Ha); 4.04 m, 1 H (H-2′); 4.23 dd, 1 H, *J*(1′b,2′) = 7.3, *J*(gem) = 14.4 (Hb-1′); 4.27 dd, 1 H, *J*(1′a,2′) = 4.4, *J*(gem) = 14.4 (Ha-1′); 4.44 dsept, 1 H and 4.47 dsept, 1 H, *J*(CH,CH_3) = 6.1, *J*(P,OCH) = 7.6 (2 × POCH); 4.50 d, 1 H and 4.52 d, 1 H, *J*(gem) = 12.0 (CH_2Ph); 7.08 bs, 2 H (NH_2); 7.27–7.37 m, 5 H (H-arom.); 8.26 s, 1 H (H-8).

6-(Aminomethyl)-9-(2-phosphonomethoxyethyl)purine (**11a**)

A solution of compound **9a** (380 mg, 1.04 mmol) in absolute ethanol (50 ml) was hydrogenated at 25 °C/101 kPa in the presence of 5% Pd-C (500 mg) for 48 h. The mixture was then filtered through a Celite pad and the solvent was evaporated. The amorphous residue was dried, dissolved in acetonitrile (30 ml) and, while stirring under argon atmosphere, TMSBr (1 ml, 7.6 mmol) was added and the resulting solution was allowed to stand overnight at 25 °C. The mixture was taken down in vacuo and codistilled with toluene (2 × 50 ml). The residue was dissolved in water (30 ml), aqueous ammonia (35%; 1 ml) was added, the solution was washed with ether (2 × 30 ml) and applied onto a column of Dowex 1X2 (acetate form, 50 ml). The column was washed with water and eluted with 0.01 M aqueous acetic acid. Evaporation of the appropriate fractions afforded the compound **11a** (130 mg, 44%) as amorphous glass that crystallized from aqueous ethanol on addition of ether. M.p. 161–164 °C (dec.), E_{Up} 0.67. For $C_9H_{14}N_5O_4P \cdot H_2O$ (305.2) calculated: 35.41% C, 5.28% H, 22.94% N, 10.14% P; found: 35.28% C, 5.28% H, 23.06% N, 9.95% P. Mass spectrum (FAB), *m/z* (rel.%): 288 (100) [M + H], 272 (21) [M + H – NH_2]. ^{1}H NMR spectrum (D_2O): 3.64 d, 2 H, *J*(P,CH) = 8.55 (PCH_2); 4.04 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 4.61 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.81 s, 2 H (NCH_2); 8.65 s, 1 H and 8.98 s, 1 H (H-2 and H-8). UV spectrum (water): pH 7, 265 (6 700); pH 12, 264 (6 700); pH 2, 264 (5 900).

6-(Aminomethyl)-9-(3-hydroxy-2-phosphonomethoxypropyl)purine (**11b**)

A solution of compound **9b** (800 mg, 1.64 mmol) in ethanol (150 ml) was hydrogenated in the same manner as described for the compound **9a** and the reaction with TMSBr (2 ml, 15.2 mmol) was performed in acetonitrile (70 ml). Anion exchange chromatography followed by preparative HPLC and freeze-drying afforded pure compound **11b** as an extremely hygroscopic violet lyophilizate (60 mg, 12%). E_{Up} 0.80. For $C_{10}H_{16}N_5O_5P \cdot x\,H_2O$ N/P found: 5.15; calculated: 5.00. Mass spectrum (FAB), *m/z* (rel.%): 318 (31) [M + H]. ^{1}H NMR spectrum (D_2O): 3.52 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.9 (PC-Hb); 3.62 dd, 1 H, *J*(3′b,2′) = 4.6, *J*(gem) = 12.5 (Hb-3′); 3.70 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (PC-Ha); 3.84 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.5 (Ha-3′); 3.95 m, 1 H (H-2′); 4.54 dd, 1 H, *J*(1′b,2′) = 7.3, *J*(gem) = 14.9 (Hb-1′); 4.64 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 14.9 (Ha-1′); 4.82 s, 2 H (NCH_2); 8.65 s, 1 H and 8.98 s, 1 H (H-2 and H-8). UV spectrum (water): pH 7, 265; pH 12, 264; pH 2, 263 nm.

2-Amino-6-(aminomethyl)-9-(2-phosphonomethoxyethyl)purine (**12a**)

A solution of compound **10a** (350 mg, 0.92 mmol) in absolute ethanol (100 ml) was hydrogenated in the presence of 5% Pd-C (200 mg) at 25 °C/101 kPa for 48 h. The mixture was then filtered through a Celite pad and the solvent was evaporated. The residue was dissolved in water (50 ml) and filtered again. The filtrate was evaporated and dried. The residue was then dissolved in a mixture of DMF (10 ml) and acetonitrile (30 ml) and TMSBr (3 ml, 22.8 mmol) was added under stirring in an argon atmosphere. The stirring was continued for 2 h and the solution was allowed to stand overnight at 25 °C. The mixture was taken down in vacuo and codistilled with toluene (2 × 50 ml). The residue was dissolved in water (30 ml), aqueous ammonia (35%; 1 ml) was added, the solution was washed with ether (2 × 50 ml) and applied onto a column of Dowex 50X8 (H^+ form, 50 ml), washed with water and

eluted with 5% aqueous ammonia. After evaporation of the appropriate UV absorbing fraction the residue was applied to a column of Dowex 1X2 (acetate form, 50 ml), the column was washed with water and the compound was eluted with 0.01 M aqueous acetic acid. Preparative HPLC of the residue (110 mg) after evaporation of the appropriate fractions followed by freeze-drying afforded the compound **12a** as a greenish hygroscopic lyophilizate (80 mg, 30%). E_{Up} 0.66. For $C_9H_{15}N_6O_4P$. H_2O (320.3) calculated: 33.75% C, 5.35% H, 26.24% N, 9.67% P; found: 34.12% C, 5.41% H, 26.04% N, 9.35% P. Mass spectrum (FAB), *m/z* (rel.%): 303 (32) [M + H], 185 (100). ^{1}H NMR spectrum (D_2O): 3.62 d, 2 H, *J*(P,CH) = 8.6 (PCH_2); 3.96 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 4.37 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.56 s, 2 H (NCH_2); 8.21 s, 1 H (H-8). UV spectrum (water): pH 7, 306 (6 000), 245 (3 200); pH 12, 304 (6 200), 244 (3 400); pH 2, 317 (5 000), 237 sh (3 900).

2-Amino-6-(aminomethyl)-9-(3-hydroxy-2-phosphonomethoxypropyl)purine (**12b**)
and 2-Amino-6-(aminomethyl)-9-(3-benzyloxy-2-phosphonomethoxypropyl)purine (**12c**)

Compound **10b** (850 mg, 1.7 mmol) was hydrogenated in ethanol (150 ml) over 5% Pd-C (500 mg) as described for compound **10a** and the intermediate was treated with TMSBr (5 ml, 38 mmol) in acetonitrile (50 ml). The ion exchange chromatography followed by preparative HPLC and freeze drying afforded compound **12b** as a greenish hygroscopic lyophilizate (120 mg, 21%). E_{Up} 0.67. For $C_{10}H_{17}N_6O_5P$. 2 H_2O (368.3) calculated: 32.61% C, 5.75% H, 22.82% N, 8.41% P; found: 32.87% C, 5.09% H, 22.54% N, 8.27% P. Mass spectrum (FAB), *m/z* (rel.%): 333 (18) [M + H], 243 (100). ^{1}H NMR spectrum (D_2O): 3.38 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.9 (PC-Hb); 3.44 dd, 1 H, *J*(3′b,2′) = 4.9, *J*(gem) = 12.5 (Hb-3′); 3.53 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (PC-Ha); 3.64 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.5 (Ha-3′); 3.74 m, 1 H (H-2′); 4.15 dd, 1 H, *J*(1′b,2′) = 7.1, *J*(gem) = 14.9 (Hb-1′); 4.25 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 14.9 (Ha-1′); 4.42 s, 2 H (NCH_2); 8.04 s, 1 H (H-8). UV spectrum (water): pH 7, 307 (4 800), 243 (1 800); pH 12, 303 (5 100), 243 (2 300); pH 2, 317 (3 600), 236 sh (2 200). Further elution of the HPLC column afforded the 3-*O*-benzyl derivative **12c** (50 mg, 7%) as amorphous solid. E_{Up} 0.61. Exact mass (FAB HRMS) for $C_{17}H_{24}N_6O_5P$ [M + H] calculated: 423.1546, found: 423.1541. ^{1}H NMR spectrum (D_2O): 3.43 dd, 1 H, *J*(3′b,2′) = 4.2, *J*(gem) = 11.0 (Hb-3′); 3.48 dd, 1 H, *J*(P,CHb) = 10.0, *J*(gem) = 12.9 (PC-Hb); 3.54 dd, 1 H, *J*(3′a,2′) = 4.6, *J*(gem) = 11.0 (Ha-3′); 3.61 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 12.9 (PC-Ha); 3.84 m, 1 H (H-2′); 4.18 dd, 1 H, *J*(1′b,2′) = 5.9, *J*(gem) = 14.9 (Hb-1′); 4.22 dd, 1 H, *J*(1′a,2′) = 4.6, *J*(gem) = 14.9 (Ha-1′); 4.30 d, 1 H and 4.34 d, 1 H, *J*(gem) = 12.0 (CH_2Ph); 4.31 d, 1 H and 4.37 d, 1 H, *J*(gem) = 16.4 (NCH_2); 7.11–7.21 m, 5 H (H-arom); 7.95 s, 1 H (H-8).

6-Carboxamidinio-9-(2-hydrogenphosphonatomethoxyethyl)purine (**14a**)

To a stirred solution of compound **9a** (250 mg, 0.68 mmol) in absolute methanol (80 ml) 1 M sodium methoxide solution (68 µl, 0.068 mmol) was added and the solution was allowed to stand for 2 days at 20 °C (TLC showed almost quantitative conversion). Ammonium chloride (100 mg, 1.8 mmol) was added and the solution was refluxed for 2 h. Evaporation of the solvent gave the crude amidinium chloride **13a** as yellow hygroscopic powder (containing some NH_4Cl and NaCl). Exact mass (FAB HRMS) found: 385.1780 (100); for $C_{15}H_{26}N_6O_4P$ [M – Cl] calculated: 385.1753. ^{1}H NMR spectrum ($(CD_3)SO$): 1.09 d, 6 H, and 1.15 d, 6 H, *J*(CH_3,CH) = 6.1 (4 × CH_3); 3.81 d, 2 H, *J*(P,CH_2) = 8.3 (PCH_2); 4.01 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 4.46 dsept, 2 H, *J*(CH,CH_3) = 6.1, *J*(P,OCH) = 7.8 (2 × POCH); 4.62 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 7.90 bs, 15 H (NH + NH_4Cl); 9.08 s, 1 H and 9.14 s, 1 H (H-2 and H-8). This compound was without purification dissolved in a mixture of acetonitrile (80 ml) and DMF (5 ml), filtered and the filtrate was treated with TMSBr (4 ml, 30 mmol) under stirring. The solution was set aside for 2 days at 20 °C, the solvents were evaporated and the residue dissolved in water (20 ml). Aqueous ammonia (35%; 1 ml) was added, the solution was

washed with ether (2 × 50 ml) and evaporated. The residue was dissolved in water (5 ml) and after addition of 35% HCl (0.2 ml) applied onto a column of Dowex 50X8 (H^+ form, 50 ml) which was washed with water to drop of UV absorption and then with 1% ammonia. The UV-absorbing fraction of the ammonia eluate was evaporated, the residue dissolved in water (5 ml) and after addition of 35% ammonia (1 ml) applied onto a column of Dowex 1X2 (acetate form, 50 ml). The column was washed with water and the product eluted with 0.01 M acetic acid. Evaporation of the UV-absorbing fraction afforded the amidine **14a** as a brownish hygroscopic powder (90 mg, 44%), m.p. 239–242 °C (dec.), E_{Up} 0.62. For $C_9H_{13}N_6O_4P$. 1.75 H_2O (331.7) calculated: 32.58%C, 5.01% H, 25.33% N; found: 32.51% C, 4.40% H, 25.59% N. Mass spectrum (FAB), *m/z* (rel.%): 301 (28) [M + H], 57 (100). ^{1}H NMR spectrum (D_2O): 3.69 d, 2 H, *J*(P,CH) = 8.8 (PCH_2); 4.07 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 4.66 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 8.84 s, 1 H and 9.10 s, 1 H (H-2 and H-8). UV spectrum (water): pH 7, 296 (8 100), 249 sh (3 600); pH 12, 279 (9 700); pH 2, 294 (7 700), 250 sh (3 900).

2-Amino-6-carboxamidinio-9-(2-hydrogenphosphonatomethoxyethyl)purine (**14b**)

To a solution of compound **10a** in absolute methanol (120 ml) was added 1 M sodium methoxide solution (150 µl, 0.15 mmol) and the solution was stirred for 2 days at 65 °C (TLC – almost quantitative conversion). Ammonium chloride (250 mg, 4.5 mmol) was added and the stirring at 65 °C was continued for 5 h. The solvent was evaporated to give the crude amidinium chloride **13b** as a yellow hygroscopic powder (contaminated with NH_4Cl and NaCl). Exact mass (FAB HRMS) found: 400.1885 (100); for $C_{15}H_{27}N_7O_4P$ $[M - Cl]^+$ calculated: 400.1862. ^{1}H NMR spectrum ($(CD_3)SO$): 1.10 d, 6 H and 1.15 d, 6 H, *J*(CH_3,CH) = 6.1 (4 × CH_3); 3.78 d, 2 H, *J*(P,CH) = 8.3 (PCH_2); 3.90 t, 2 H, *J*(2′,1′)= 5.0 (H-2′); 4.32 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.47 dsept, 2 H, *J*(CH,CH_3) = 6.1, *J*(P, OCH) = 7.6 (POCH); 6.95 bs, 2 H (NH_2); 8.00 bs, 10 H (NH + NH_4Cl); 8.44 s, 1 H (H-8). This material was dissolved without purification in a mixture of DMF (100 ml) and acetonitrile (50 ml), filtered and the filtrate was treated with TMSBr (10 ml, 76 mmol) as described for compound **13a**. Analogous isolation by ion exchange chromatography afforded the amidine **14b** as a yellow powder, m.p. 298–300 °C (dec.), yield 240 mg (51%). E_{Up} 0.54. For $C_9H_{14}N_7O_4P$ (315.2) calculated: 34.29% C, 4.48% H, 31.10% N, 9.83% P; found: 34.07% C, 4.41% H, 30.86% N, 9.49% P. Mass spectrum (FAB), *m/z* (rel.%): 316 (100) [M + H]. ^{1}H NMR spectrum (D_2O): 3.64 d, 2 H, *J*(P,CH) = 8.5 (PCH_2); 4.04 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 4.37 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 8.39 s, 1 H (H-8). UV spectrum (water): pH 7, 361 (6 200), 277 (1 000); pH 12, 334 (6 900), 266 (3 600); pH 2, 360 (6 300), 275 sh (1 100).

Antiviral Activity Assays

The antiviral activity assays other than for HIV-1 were based on inhibition of virus-induced cytopathicity in either E_6SM or HEL cell cultures, following the previously established procedures[15,16]. Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of the virus (1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures). After a 1 h virus adsorption period, the residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... µg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Inhibition of HIV-1-Induced Cytopathicity in MT-4 Cells

The methodology of the anti-HIV activity assays has been described previously[17]. Briefly, human MT-4 cells (5 . 10^5 cell/ml) were infected with 100 $CCID_{50}$ HIV-1 (strain HTLV-III_B)/ml and seeded in 200 µl-wells of a microtiter plate, containing the test compounds in appropriate dilutions. After

5 days of incubation at 37 °C, the number of viable cells was determined in a blood cell counting chamber by trypan blue dye exclusion.

Inhibition of MSV-Induced Transformation of Murine C3H/3T3 Embryo Fibroblasts

The anti-MSV activity assay was performed as described previously[18]. Murine C3H/3T3 embryo fibroblast cells were seeded at $5 . 10^5$ cell/ml into 1 cm^2 wells of a 48-well microplate. After 24 h, the cell cultures were infected with 80 focus-forming units of MSV (prepared from tumours induced following intramuscular inoculation of 3 days old NMRI mice with MSV, as described previously[19]) for 60–90 min at 37 °C. The medium was then replaced by 1 ml of fresh medium containing various concentrations of the test compounds. After 6 days, the transformation of the cell culture was examined microscopically.

This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (Grants No. 455402 and No. 455407), by the Grant Agency of the Czech Republic (Grant No. 203/93/0118), by the PECO project No. ERBCIPDCT 940202 of the European Commission, by the Grants from the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek No. 3.3010.91, the Belgian Genconcerteerde Onderzoeksacties No. 95/5 and by Gilead Sciences (Foster City, CA, U.S.A.). The cytostatic activity was studied by Dr I. Votruba at the Institute of Organic Chemistry and Biochemistry. The authors' thanks are due to Mr J. Günter for performing the preparative HPLC, to the staff of mass spectrometry and analytical departments and to Mrs A. Absillis, L. van Berckelaer, A. Van Lierde, F. De Meyer and A. Camps for technical assistance with the antiviral activity assays.

REFERENCES

1. a) Holy A. in: *Isopolar Phosphorus-Modified Nucleotide Analogues in Advances in Antiviral Drug Design* (E. De Clercq, Ed.), Vol. 1, p. 179. JAI Press Inc., Greenwich (U.S.A.)/London 1993. b) Holy A., Dvorakova H., Jindrich J. in: *Antiviral Acyclic Nucleotide Analogues in Antibiotics and Antiviral Compounds* (K. Krohn, H. A. Kirst and H. Maag, Eds), p. 455. Verlag Chemie, Berlin/Heidelberg 1993. c) Vesely J., Vlach J., Sediva K., Rosenberg I., Otmar M., Hrebabecky H., Travnicek M., Vonka V., Snoeck R., De Clercq E.: Antiviral Res. *13*, 295 (1990).
2. Holy A., Snoeck R., Andrei G., Balzarini J., De Clercq E.: Antiviral Res. *26*, A231 (1995).
3. Hocek M., Masojidkova M., Holy A.: Collect. Czech. Chem. Commun. *60*, 875 (1995).
4. Giner-Sorolla A., Bendich A.: J. Am. Chem. Soc. *80*, 3932 (1958).
5. Herdewijn P., Van Aerschot A., Pfleiderer W.: Synthesis *1989*, 961.
6. Hocek M., Holy A.: Collect. Czech. Chem. Commun. *60*, 1386 (1995).
7. Dvorakova H., Holy A.: Collect. Czech. Chem. Commun. *58*, 1419 (1993).
8. Bronson J. J., Ghazzouli I., Hitchcock M. J. M., Webb R. R., Martin J. C.: J. Med. Chem. *32*, 1457 (1989).
9. Holy A.: Collect. Czech. Chem. Commun. *58*, 649 (1993).
10. Hockova D., Masojidkova M., Budesinsky M., Holy A.: Collect. Czech. Chem. Commun. *60*, 224 (1995).
11. Sajiki H.: Tetrahedron Lett. *36*, 3465 (1995).
12. Phelan M. J., Gabrielsen B., Kirsi J. J., Shannon W. M., Ussery M. A., Barthel-Rosa L., Schubert E. M., Kini G. D., Robins R. K.: Nucleosides Nucleotides *14*, 1315 (1995).
13. Thorpe M. C., Coburn W. C., Jr., Montgomery J. A.: J. Magn. Reson. *15*, 98 (1974).
14. Lok C. M.: Chem. Phys. Lipids *22*, 323 (1978).

15. De Clercq E., Descamps J., Verhelst G., Walker R. T., Jones A. S., Torrence P. F., Shugar D.: J. Infect. Dis. *141*, 563 (1980).
16. De Clercq E., Holy A., Rosenberg I., Sakuma T., Balzarini J., Maudgal P. C.: Nature *323*, 464 (1986).
17. Balzarini J., Naesens L., Slachmuylders J., Niphuis H., Rosenberg I., Holy A., Schellekens H., De Clercq E.: AIDS *5*, 21 (1991).
18. Balzarini J., Naesens L., Herdewijn P., Rosenberg I., Holy A., Pauwels R., Baba M., Johns D. G., De Clercq E.: Proc. Natl. Acad. Sci. U.S.A. *86*, 332 (1989).
19. De Clercq E., Merigan T. C.: Proc. Soc. Exp. Biol. Med. *137*, 590 (1971).