Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 3197–3202
Bioorganic & Medicinal Chemistry

Synthesis and antiviral activity of 2,4-diamino-5-cyano-6-[2-(phosphonomethoxy)ethoxy]pyrimidine and related compounds

Dana Hocková,[a,*] Antonín Holý,[a] Milena Masojídková,[a] Graciela Andrei,[b] Robert Snoeck,[b] Erik De Clercq[b] and Jan Balzarini[b]

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-166 10 Prague 6, Czech Republic*
[b]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Received 22 December 2003; accepted 2 April 2004
Available online 8 May 2004

Abstract—Synthesis of 2,4-diamino-5-cyano-6-{[(diisopropoxyphosphoryl)methoxy]ethoxy}pyrimidine was based on the formation of the pyrimidine ring by cyclization followed by modification of the side chain by alkylation. The 5-cyano group was also transformed to a 5-formyl and 5-hydroxymethyl group by reduction. As a side product an unexpected dimer was formed. Resulting compounds were converted to the free phosphonic acids by treatment with bromotrimethylsilane followed by hydrolysis. The 5-cyano and 5-formyl derivatives showed pronounced antiretroviral activity, comparable to that of the reference drugs adefovir and tenofovir.

1. Introduction

2-(Phosphonomethoxy)alkyl derivatives of purine and pyrimidine bases—acyclic nucleoside phosphonates (ANPs)—possess significant antiviral and cytostatic activity.[1] These nucleotide analogues contain an isopolar phosphonomethyl ether moiety instead of the nucleotide phosphate ester group, which excludes their enzymatic degradation and/or eliminates problems with intracellular phosphorylation necessary for nucleoside activation. Among the ANPs, particularly 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir, Fig. 1) and [(*R*)-2-phosphonomethoxypropyl]adenine ((*R*)-PMPA, tenofovir, Fig. 1) are active against DNA viruses and retroviruses.[2] In this paper, these commercially available drugs are included as reference compounds for the antiviral evaluation assays.

The SAR studies demonstrated that the margins of structural alteration are very narrow.[3] Except for the antiviral activity of the cytosine derivative (*S*)-HPMPC (cidofovir, Fig. 1),[4] the choice of the base is limited mostly to adenine, guanine, or 2,6-diaminopurine, and to their 8-aza and 3-deaza congeners.[5] The pharmacophore of purine acyclic nucleoside phosphonates is characterized by the presence of amino group(s) at the pyrimidine part of the purine system.

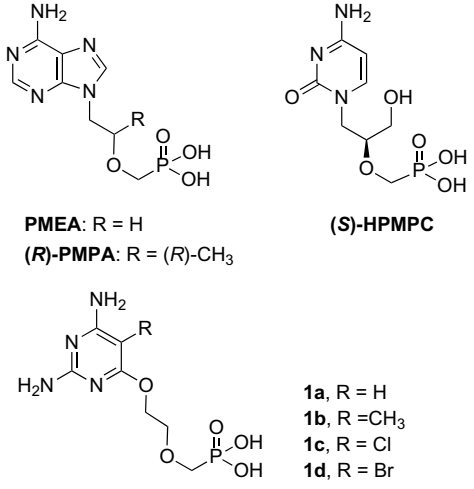


Figure 1.

Recently we discovered a new type of antiviral ANPs originating from 2-substituted 4-amino-6-hydroxypyrimidines.[6]

Keywords: Nucleotide analogues; Acyclic nucleoside phosphonates; Antiviral; Pyrimidines.
* Corresponding author. Tel.: +420-220183262; fax: +420-220183560; e-mail: lasice@uochb.cas.cz

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.04.002

Alkylation of these bases by the appropriate phosphonate-protected synthon afforded a mixture of O6- and N1-regioisomers, which were converted to the free phosphonates. While none of the isomeric 1-[2-(phosphonomethoxy)ethyl]pyrimidin-6-one derivatives was antivirally active, among 6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives, compounds derived from 2,4-diaminopyrimidine significantly inhibited replication of retroviruses and herpesviruses in cell culture. These novel subclass of pyrimidine ANPs can be considered as analogues of 2,6-diaminopurine with an open imidazole ring in the purine moiety.

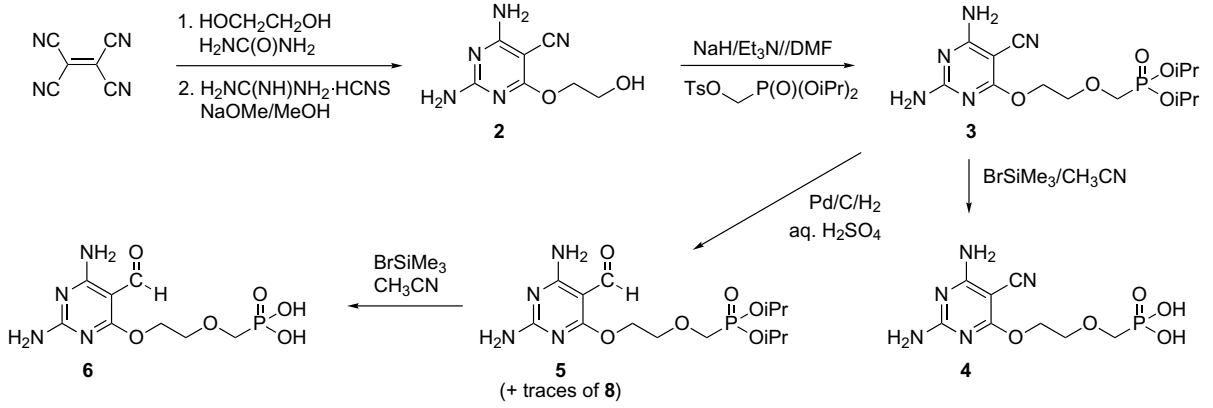
To further investigate the structure–activity relationship in this new group of antiviral compounds, recently we reported[7] the synthesis and antiviral activity of 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives (**1**, Fig. 1) bearing various substituents at the C5-position. In this case, the imidazole ring of the purine base is even more closely mimicked. Among these compounds, the 5-methyl derivative **1b** was markedly active against human immunodeficiency virus [at least 10-fold more than the 5-unsubstituted compound (**1a**)] and Moloney murine sarcoma virus-induced cytopathicity in cell culture (EC_{50}: ~0.00018 μmol/mL) but also cytostatic to CEM cell cultures. In contrast, the 5-halogen-substituted derivatives **1c** and **1d** showed pronounced antiretroviral activity (EC_{50}: 0.0023–0.0110 μmol/mL), comparable to that of the reference drugs adefovir and tenofovir, but were devoid of measurable toxicity at 0.3 μmol/mL.

Since several 5-substituted 2,4-diaminopyrimidine derivatives markedly inhibited retrovirus replication in cell culture, we decided to further enlarge the diversity of the functional groups at the 5-position of pyrimidine moiety.

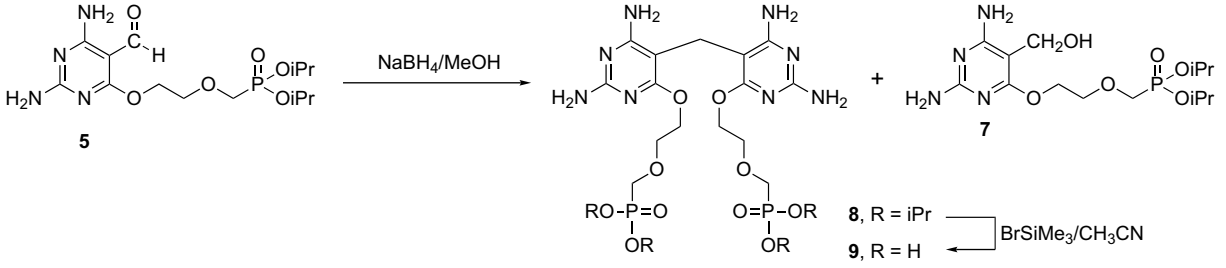
2. Results and discussion

As a suitable parent compound 2,4-diamino-5-cyano-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**3**) was selected. For the creation of its intermediate 2,4-diamino-5-cyano-6-(2-hydroxyethoxy)pyrimidine (**2**) a known two-step approach starting from tetracyanoethylene was applied.[8] The second step is based on the formation of pyrimidine ring by the cyclization of dicyanoketene ethylene acetal with guanidine.[9] The hydroxy group in the side chain of compound **2** was alkylated by (diisopropoxyphosphoryl)methyl tosylate[7] (Scheme 1) to form the desired 2,4-diamino-5-cyano-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**3**). This alkylation reaction is aggravated by the insolubility of starting material in THF and so the standard conditions had to be optimized.

The cyano group at the 5-position was further modified by reduction. The synthesis of 5-formyl derivative **5** was achieved by hydrogenation of compound **3** in a mixture of water and sulfuric acid over palladium on charcoal. The formyl group underwent consecutive reduction (Scheme 2) by sodium borohydride in methanol to afford 5-(hydroxymethyl)pyrimidine **7**. This compound was rather unstable and when the reaction time of the reduction was prolonged, only an unexpected product **8** was isolated instead of the compound **7**. Traces of the same product were isolated also from the hydrogenation



Scheme 1.



Scheme 2.

of 5-cyano derivative **3** when the reaction mixture was allowed to react for three days.

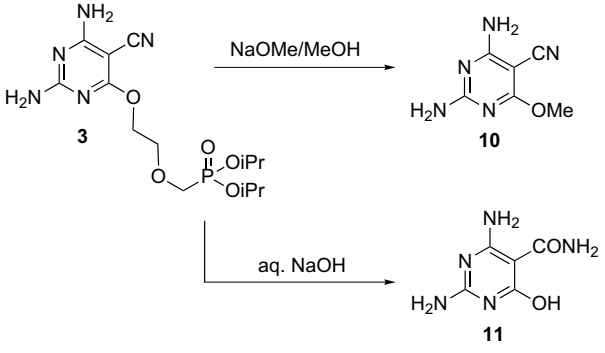
2,4-Diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidines bearing 5-cyano and 5-formyl groups (compounds **3** and **5**) smoothly afforded, on treatment with bromotrimethylsilane followed by hydrolysis, the free phosphonic acids **4** and **6**, that were purified by ion-exchange column chromatography on Dowex 1×2 (OAc^-). In contrast, the 5-hydroxymethyl compound **7** afforded no desired product under the above reaction conditions or under the modified conditions in the presence of lutidine.[11]

The same standard procedure was used for deprotection of both the phosphono diester groups in dimer **8** to form bisphosphonic acid **9**. In ^{1}H NMR spectrum of compound **9** a two-proton singlet (δ 3.40) was found together with two four-proton multiplets of H-1′ and H-2′ and four-proton doublet of P-CH_2 (see Experimental). In ^{13}C NMR spectrum (APT), except for signals assigned to carbons of the pyrimidine base and a side chain, also a signal of the methylene group was observed (δ 15.28). These results together with the mass spectrum of derivative **9** are in good agreement with suggested structure. Formation of the dimer during the reduction is caused probably by the reaction of 5-unsubstituted side product with 5-hydroxymethyl derivative **7**. Similar type of dimers linked by methylene group has been observed previously.[10]

Our attempts to modify the cyano group at the 5-position by hydrolysis failed. Acidic hydrolysis of **3** in refluxing aqueous H_2SO_4 gave a very complex reaction mixture. At room temperature and lower acid concentration only starting compound was isolated. Under basic conditions the side chain attached via an ether bond at the 6-position was cleaved. When sodium methoxide in methanol was used, only 2,4-diamino-5-cyano-6-methoxypyrimidine (**10**) was isolated. Hydrolysis in aqueous NaOH afforded compound **11** (Scheme 3).

2.1. Biological activity

The 5-cyano and 5-formyl 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidines (**4** and **6**) and the bisphosphonic acid **9** were investigated for their inhibitory



Scheme 3.

Table 1. Antiviral activity of test compounds in cell culture

Example	EC_{50}[a] (μmol/mL)			CC_{50}[b] (μmol/mL) (CEM)
	HIV-1 (III_B)	HIV-2 (ROD)	MSV	
4 (5-CN)	0.011	0.0045	0.0095	⩾ 0.3
6 (5-CHO)	0.0045	0.0027	0.021	⩾ 0.3
9	0.080	0.050	—	⩾ 0.2
Reference compounds				
1a (5-H)	0.0031	0.0016	0.00015	0.042
1b (5-CH_3)	0.00023	0.00023	0.00016	0.0047
PMEA	0.0033	0.0066	0.0022	0.056
(*R*)-PMPA	0.0012	0.0014	0.0046	0.41

[a] 50% effective concentration.
[b] 50% cytostatic concentration.

activity against several DNA and retroviruses. In contrast to the parent 5-unsubstituted compound **1a**, none of the derivatives **4**, **6**, and **9** were active against herpes simplex virus type 1 or type 2, cytomegalovirus and vaccinia virus. Compounds **4** and **6** were inhibitory against HIV at 0.0027–0.011 μmol/mL, and against MSV at 0.0095–0.021 μmol/mL (Table 1). These antiviral potencies were comparable with those found for the 5-unsubstituted 2,4-diamino-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine against HIV but inferior the values found for MSV. These values were also comparable to those recorded for adefovir and tenofovir. Compared with the 5-methyl derivative **1b**, the antiretroviral activities were lower. Compound **9** was 10- to 20-fold less inhibitory to HIV than **4** and **6**. The compounds showed no appreciable cytotoxic activity against E_6SM, HEL, or CEM cell growth and did not affect microscopically visible cell morphology.

3. Experimental

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on an FT NMR spectrometer Varian UNITY 500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz frequency) in dimethyl sulfoxide-d_6 or D_2O. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å).

3.1. 2,4-Diamino-5-cyano-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (3)

Starting 2,4-diamino-5-cyano-6-(2-hydroxyethoxy)pyrimidine (**2**) was prepared in two steps from tetracyanoethylene according to the literature, yield 73%.[8,9] For $C_7H_9N_5O_2$ (195.2) calcd: C, 43.08; H, 4.65; N, 35.88. Found: C, 42.84; H, 4.71; N, 35.55. FABMS: 196 [MH^+] (20). ^{1}H NMR (DMSO-d_6): 6.94 (br s, 2H) and 6.88 (br s, 2H) (NH_2); 4.83 (t, 1H, J(OH,2′) = 5.2, OH); 4.28 (t, 2H, J(1′,2′) = 5.4, H-1′); 3.65 (q, 2H, J(1′,2′) =

J(OH,2′) = 5.3, H-2′). ^{13}C NMR: 171.14 (C-6); 165.99 (C-4); 163.06 (C-2); 116.27 (CN); 67.86 (C-5); 63.09 (C-1′); 59.37 (C-2′). To the suspension of this compound (2.4 g, 12 mmol) in triethylamine (8 mL) (diisopropoxyphosphoryl)methyl tosylate (4.8 g, 13.7 mmol) was added followed by dimethylformamide (20 mL) and NaH (60% disp. in mineral oil, 1.3 g). The reaction mixture was stirred at room temperature for 1 h, evaporated in vacuo and codistilled successively with toluene and ethanol. The residue was chromatographed on silica gel. The fractions eluted with 4% MeOH in $CHCl_3$ gave 2 g (45%) of the product, that was without further purification used in the next step. FABMS: 374 [MH^+] (10), 152 [baseH^+] (100). ^{1}H NMR (DMSO-d_6): 6.95 (br s, 2H) and 6.90 (br s, 2H) (NH_2); 4.59 (m, 2H, P-OCH); 4.40 (m, 2H, H-1′); 3.81 (d, 2H, J(P,CH) = 8.3, P-CH_2); 3.80 (m, 2H, H-2′); 1.24 (d, 6H) and 1.23 (d, 6H) (CH_3). ^{13}C NMR: 170.86 (C-6); 165.95 (C-4); 162.99 (C-2); 116.05 (CN); 70.61 (d, J(P,C) = 11.7, C-2′); 70.41 (d, J(P,C) = 6.3, P-OC); 65.15 (C-5); 65.12 (d, J(P,C) = 164.1, P-C); 63.03 (C-1′); 24.01 (d, 2C, J(P,C) = 3.9) and 23.88 (d, 2C, J(P,C) = 4.4) (CH_3).

3.2. 2,4-Diamino-5-cyano-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (4)

2,4-Diamino-5-cyano-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**3**, 0.19 g, 0.5 mmol), acetonitrile (10 mL), and $BrSiMe_3$ (1 mL) was stirred overnight at room temperature. After evaporation in vacuo and codistillation with acetonitrile, the residue was treated with water and concd aqueous ammonia was added to alkaline reaction. The mixture was evaporated to dryness and the residue was applied onto column of Dowex 50×8 (H^+-form, 20 mL) and washed with water. Elution with 2.5% aqueous ammonia and evaporation in vacuo afforded crude product as ammonium salt. This residue in minimum volume of water was applied on Dowex 1×2 (acetate, 25 mL) column, which was then washed with water followed by gradient of acetic acid (0–3 M). The main UV absorbing fraction was evaporated, the residue was three-times codistilled with water and crystallized from water–ethanol to afford product as a white solid: (120 mg, 86%), mp 294–295 °C. For $C_8H_{12}N_5O_5P{\cdot}H_2O$ (307.2) calcd: C, 31.28; H, 4.59; N, 22.80; P, 10.08. Found: C, 31.27; H, 4.67; N, 22.54; P, 9.94. FABMS: 290 (MH^+) (10). ^{1}H NMR (D_2O+NaOD): 4.48 (m, 2H, H-1′); 3.95 (m, 2H, H-2′); 3.74 (d, 2H, J(P,CH) = 8.4, P-CH_2). ^{13}C NMR: 171.87 (C-6); 166.15 (C-4); 163.30 (C-2); 116.85 (CN); 70.88 (d, J(P,C) = 10.7, C-2′); 67.69 (d, J(P,C) = 156.2, P-C); 66.72 (C-5); 64.52 (C-1′).

3.3. 2,4-Diamino-5-formyl-6-[2-(diisopropoxyphosphoryl-methoxy)ethoxy]pyrimidine (5)

5-Cyano-2,4-diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**3**, 1.5 g, 4 mmol) was hydrogenated in water (36 mL) and sulfuric acid (6 mL) over 5% palladium on charcoal (0.25 g) under stirring for 20 h at room temperature. The mixture was filtered through a pad of Celite and the catalyst was washed with hot water and hot methanol (100 mL each). The filtrate was neutralized with aqueous NaOH and solvents evaporated. The residue was mixed with hot methanol, salts were filtered off, and the mixture was taken down. Product was purified by column chromatography on silica gel (elution with 3% MeOH in $CHCl_3$) to give 0.63 g (42%) of the desired product (also 0.11 g (4%) of compound **8** was isolated as a side product). FABMS: 377 (MH^+) (10). ^{1}H NMR (DMSO-d_6): 9.82 (s, 1H, CHO); 8.30 (br d, 1H), 7.44 (br d, 1H, J = 4.0), and 7.09 (br s, 2H) (NH_2); 4.58 (m, 2H, P-OCH); 4.43 (m, 2H, H-1′); 3.83 (m, 2H, H-2′); 3.81 (d, 2H, J(P,CH) = 8.3, P-CH_2); 1.22 (d, 6H) and 1.21 (d, 6H, J = 6.2) (CH_3). ^{13}C NMR: 184.77 (CHO); 171.50 (C-6); 164.62 (C-4); 163.91 (C-2); 91.84 (C-5); 70.69 (d, J(P,C) = 12.2, C-2′); 70.32 (d, J(P,C) = 6.3, P-OC); 65.04 (d, J(P,C) = 164.6, P-C); 64.83 (C-1′); 23.95 (d, 2C, J(P,C) = 3.4) and 23.82 (d, 2C, J(P,C) = 4.4) (CH_3).

3.4. 2,4-Diamino-5-formyl-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (6)

5-Formyl-2,4-diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**5**, 0.36 g, 0.95 mmol), acetonitrile (10 mL), and $BrSiMe_3$ (1 mL) was stirred for 2.5 h at room temperature. After evaporation in vacuo and codistillation with acetonitrile, the residue was treated with water and triethylamine was added to alkaline reaction. The mixture was evaporated to dryness and the residue was applied onto column of Dowex 1×2 (acetate, 25 mL) column, which was then washed with water followed by gradient of acetic acid (0–2 M, 1 L each). The main UV absorbing fraction was evaporated, the residue was three-times codistilled with water and crystallized from water–methanol to afford the product as a white solid: (230 mg, 82%), mp 225–227 °C. For $C_8H_{13}N_4O_6P{\cdot}5/4H_2O$ (314.7) calcd: C, 30.53; H, 4.96; N, 17.80. Found: C, 30.87; H, 5.01; N, 17.65. FABMS: 293 (MH^+) (10). ^{1}H NMR (D_2O+NaOD): 9.73 (s, 1H, CH=O); 4.46 (m, 2H, H-1′); 3.95 (m, 2H, H-2′); 3.59 (d, 2H, J(P,CH) = 8.4, P-CH_2). ^{13}C NMR: 187.89 (CH=O); 172.46 (C-6); 164.285 (C-4); 163.85 (C-2); 92.44 (C-5); 70.25 (d, J(P,C) = 9.3, C-2′); 69.18 (d, J(P,C) = 148.4, P-C); 66.11 (C-1′).

3.5. 2,4-Diamino-5-hydroxymethyl-6-[2-(diisopropoxy-phosphorylmethoxy)ethoxy]pyrimidine (7)

To the solution of compound **5** (0.27 g, 0.7 mmol) in methanol (5 mL) $NaBH_4$ (32 mg, 0.9 mmol) was added in portions. The reaction mixture was stirred at room temperature for 30 min, applied on short column of silica gel and washed with 40% MeOH in $CHCl_3$. Solvents were evaporated to obtain crude product as a sticky colorless foam (0.24, 89%). The product appeared to be rather unstable (in further attempts to purify compound **7** dimer **8** was isolated). FABMS: 379 [MH^+] (15). ^{1}H NMR (DMSO-d_6): 5.88 (br s, 2H) and 5.85 (br s, 2H) (NH_2); 4.59 (m, 2H, P-OCH); 4.45 (br s, 1H, OH); 4.27 (br s, 2H, CH_2OH); 4.26 (m, 2H, H-1′); 3.80

(d, 2H, J(P,CH) = 8.3, P-CH_2); 3.77 (m, 2H, H-2′); 1.24 (d, 6H) and 1.23 (d, 6H, J = 6.2) (CH_3). ^{13}C NMR: 166.55 (C-6); 164.80 (C-4); 161.59 (C-2); 89.13 (C-5); 71.18 (d, J(P,C) = 11.7, C-2′); 70.35 (d, J(P,C) = 5.8, P-OC); 65.15 (d, J(P,C) = 164.1, P-C); 64.11 (C-1′); 52.88 (CH_2OH); 24.00 (d, 2C, J(P,C) = 3.4) and 23.87 (d, 2C, J(P,C) = 4.4) (CH_3).

3.6. 5,5′-Methylenebis{2,4-diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine} (8)

The same reaction procedure as described for compound **7** was applied, but the reaction time was longer (3 h), a small amount of water was added and the reaction mixture was evaporated to dryness. Preparative TLC followed by preparative HPLC afforded compound **8** as a white solid (90 mg, 36%). FABMS: 709.5 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 5.97 (br s, 4H) and 5.81 (br s, 4H) (NH_2); 4.33 (m, 4H, H-1′); 3.83 (m, 4H, H-2′); 3.82 (d, 4H, J(P,CH) = 8.6, P-CH_2); 4.62 (m, 4H, P-OCH); 3.42 (s, 2H, CH_2); 1.25 (d, 12H) and 1.24 (d, 12H, J = 6.2) (CH_3).

3.7. 5,5′-Methylenebis{2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine} (9)

Compound **8** (0.28 g, 0.4 mmol), acetonitrile (10 mL), and $BrSiMe_3$ (1 mL) were stirred at room temperature for 20 h. After evaporation in vacuo and codistillation with acetonitrile, the residue was treated with water and triethylamine was added to alkaline reaction. The mixture was evaporated to dryness and the residue was applied onto column of Dowex 1×2 (acetate, 25 mL) column, which was then washed with water followed by gradient of acetic acid (0–2 M, 1 L each) and hot formic acid (2 M, the product was very unsoluble). Combined fractions containing product were evaporated, the residue was three-times codistilled with water and crystallized from water–methanol to afford product as a white solid: (110 mg, 51%). FABMS: 541 (MH^+) (40). For $C_{15}H_{26}N_8O_{10}P_2$ (540) calcd: C, 33.34; H, 4.85; N, 20.74. Found: C, 33.48; H, 4.98; N, 20.54. ^{1}H NMR (D_2O+NaOD): 4.45 (m, 4H, H-1′); 3.95 (m, 4H, H-2′); 3.59 (d, 4H, J(P,CH) = 8.6, P-CH_2); 3.40 (s, 2H, CH_2). ^{13}C NMR: 166.52 (C-6); 164.09 (C-4); 160.65 (C-2); 88.25 (C-5); 70.52 (d, J(P,C) = 10.7, C-2′); 69.14 (d, J(P,C) = 150.4, P-C); 65.53 (C-1′); 15.28 (CH_2).

3.8. 2,4-Diamino-5-cyano-6-methoxypyrimidine (10)

To the solution of compound **3** (0.3 g, 0.8 mmol) in methanol (8 mL) sodium methoxide (1 mL, 1 M solution in methanol) was added. The reaction mixture was stirred at room temperature for 20 h but only starting material was detected by TLC. Then the solution was refluxed for 45 h and a white solid was filtered off after cooling. This product was identified as compound **10** (0.1 g, 77%). FABMS: 166 (MH^+) (100). For $C_6H_7N_5O$ (165.2) calcd: C, 43.64; H, 4.27; N, 42.41. Found: C, 43.44; H, 4.26; N, 42.08. ^{1}H NMR (DMSO-d_6): 6.96 (br s, 4H, NH_2); 3.83 (s, 3H, OCH_3). ^{13}C NMR: 171.42 (C-6); 163.26 (C-2); 165.86 (C-4); 116.24 (CN); 62.89 (C-5); 53.76 (OCH_3).

3.9. 2,4-Diamino-6-hydroxypyrimidine-5-carboxamide (11)

Compound **3** (0.9 g, 2.4 mmol) was added to the aqueous 10% solution of NaOH (20 mL) and reaction mixture was refluxed for 3 h. Acetic acid was added to adjust pH to 6–7. After cooling to rt, white solid was filtered off and washed with methanol. This product was identified as compound **11** (0.30 g, 74%). FABMS: 152 (MH^+-H_2O) (100), 170 (MH^+) (10). For $C_5H_7N_5O_2$ (169.1) calcd: C, 35.51; H, 4.17; N, 41.41. Found: C, 35.81; H, 4.00; N, 41.79. ^{1}H NMR (DMSO-d_6): 10.46 (br s, 1H, OH), 7.05 (br s, 2H), and 6.86 (br, 2H) (NH_2). ^{13}C NMR (D_2O+NaOD): 175.66 (CO); 166.74 (C-4); 163.87 (C-2); 161.59 (C-6); 120.07 (C-5).

3.10. Antiviral activity assays

The antiviral, other than anti-HIV-1, assays were based on inhibition of virus-induced cytopathicity in either E_6SM (HSV-1, HSV-2, VV) or HEL (VZV, CMV) cell cultures, following previously established procedures. Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1- to 2-h virus adsorption period, 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 that were not treated with the test compounds.

3.11. Inhibition of HIV-1-induced cytopathicity in CEM cells

The methodology of the anti-HIV assays has been described previously.[12] Briefly, human CEM ($\sim 3\times10^5$ cells/mL) cells were infected with 100 $CCID_{50}$ HIV-1 ((III_B)) or HIV-2 (ROD) per mL and seeded in 200 μL-wells of 96-well microtiter plates, containing appropriate dilutions of the test compounds. After four days of incubation at 37 °C, CEM giant cell formation was examined microscopically.

3.12. Inhibition of MSV-induced transformation of murine C3H/3T3 embryo fibroblasts

The anti-MSV assay was performed as described previously.[12] Murine C3H/3T3 embryo fibroblast cells were seeded at 5×10^5 cells/mL into 1-cm^2 wells of 48-well microplates. Twenty four hours later, the cell cultures were infected with 80 focus-forming units of MSV (prepared from tumors induced following intramuscular inoculation of 3-day-old NMRI mice with MSV, as

described previously)[13] for 90–120 min at 37 °C. The medium was then replaced by 1 mL of fresh medium containing various concentrations of the test compounds. After six days, transformation of the cell culture was examined microscopically.

Acknowledgements

This study was performed as a part of research project # Z4055905 of the Institute of Organic Chemistry and Biochemistry. It was supported by the Programme of Targeted Projects of the Academy of Sciences of the Czech Republic (# S4055109), by Gilead Sciences (Foster City, CA, USA), by ISEP/FORTIS, the 'Geconcerteerde Onderzoeksacties' (GOA-00/12), and the René Descartes Prize-2001 of the European Commission (HPAW-2002-90001). The authors' thanks are also due to the staff of the mass spectrometry and analytical departments (Head, K. Ubik) of the Institute of Organic Chemistry and Biochemistry.

References and notes

1. Reviews: (a) Holý, A. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI: Greenwich, CT, 1994; pp 179–232; (b) Holý, A. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C. K., Ed.; Elsevier, 2002; pp 167–238.
2. (a) Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neyts, J.; De Clercq, E. *Antiviral Chem. Chemother.* **1997**, *8*, 1; (b) Ying, C.; De Clercq, E.; Neyts, J. *J. Viral Hepatitis* **2000**, *7*, 79; (c) Perrillo, R.; Schiff, E.; Yoshida, E.; Statler, A.; Hirsch, K.; Wright, T.; Gutfreund, K.; Lamy, P.; Murray, A. *Hepatology* **2000**, *32*, 129; (d) Gilson, R. J.; Chopra, K. B.; Newell, A. M.; Murray-Lyon, I. M.; Nelson, M. R.; Rice, S. J.; Tedder, R. S.; Toole, J.; Jaffe, H. S.; Weller, I. V. *J. Viral Hepatitis* **1999**, *6*, 387; (e) Srinivas, R. V.; Fridland, A. *Antimicrob. Agents Chemother.* **1998**, *42*, 1484; (f) Van Rompay, K. K.; Miller, M. D.; Marthas, M. L.; Margot, N. A.; Dailey, P. J.; Canfield, D. R.; Tarara, R. P.; Cherrington, J. M.; Aguirre, N. L.; Bischofberger, N.; Pedersen, N. C. *J. Virol.* **2000**, *74*, 1767.
3. Holý, A.; Günter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 2064.
4. For review, see: (a) De Clercq, E. *Collect. Czech. Chem. Commun.* **1998**, *63*, 480; (b) Safrin, S.; Cherrington, J.; Jaffe, H. S. *Adv. Exp. Med. Biol.* **1999**, *458*, 111; (c) De Clercq, E.; Andrei, G.; Balzarini, J.; Hatse, S.; Liekens, S.; Naesens, L.; Neyts, J.; Snoeck, R. *Nucleos. Nucleot.* **1999**, *18*, 759; (d) Snoeck, R.; Noel, J. C.; Muller, C.; De Clercq, E.; Bossens, M. *J. Med. Virol.* **2000**, *60*, 205.
5. (a) Balzarini, J.; Holý, A.; Jindřich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. *Antimicrob. Agents Chemother.* **1993**, *37*, 332; (b) Balzarini, J.; Aquaro, S.; Perno, C. F.; Witvrouw, M.; Holý, A.; De Clercq, E. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 337; (c) Naesens, L.; Neyts, J.; Balzarini, J.; Holý, A.; Rosenberg, I.; De Clercq, E. *J. Med. Virol.* **1993**, *39*, 167; (d) Kreider, J. W.; Balogh, K.; Olson, R. O.; Martin, J. C. *Antiviral Res.* **1990**, *14*, 51; (e) Yu, K. L.; Bronson, J. J.; Yang, H.; Patick, A.; Alam, M.; Brankovan, V.; Datema, R.; Hitchcock, M. J.; Martin, J. C. *J. Med. Chem.* **1992**, *35*, 2958; (f) Otová, B.; Zidek, Z.; Holý, A.; Votruba, I.; Sladká, M.; Marinov, I.; Lesková, V. *In Vivo* **1997**, *11*, 163; (g) Otová, B.; Francová, K.; Franěk, F.; Koutník, P.; Votruba, I.; Holý, A.; Sladká, M.; Schramlová, J. *Anticancer Res.* **1999**, *19*, 3173; (h) Rose, W. C.; Crosswell, A. R.; Bronson, J. J.; Martin, J. C. *J. Natl. Cancer Inst.* **1990**, *82*, 510.
6. (a) Holý, A.; Votruba, I.; Masojídková, M.; Andrei, G.; Snoeck, R.; Naesens, L.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2002**, *45*, 1918; (b) Balzarini, J.; Pannecouque, C.; De Clercq, E.; Aquaro, S.; Perno, C. F.; Egberink, H.; Holý, A. *Antimicrob. Agents Chemother.* **2002**, *46*, 2185.
7. Hocková, D.; Holý, A.; Masojídková, M.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2003**, *46*, 5064.
8. Middletton, W. J.; Engelhardt, V. A. *J. Am. Chem. Soc.* **1958**, *80*, 2788.
9. Middletton, W. J.; Engelhardt, V. A. *J. Am. Chem. Soc.* **1958**, *80*, 2829.
10. (a) Billman, J. H.; Thomas, D. G.; Hedrick, M.; Schrotenboer, G.; Barnes, D. K.; Nemec, J.; Trix, P.; Cleland, E. *J. Org. Chem.* **1946**, *11*, 773; (b) Charmantray, F.; Demeunynck, M.; Lhomme, J.; Duflos, A. *J. Org. Chem.* **2001**, *66*, 8222.
11. Alexandr, A.; Krishnamurthy, V. V.; Prisbe, E. J. *J. Med. Chem.* **1996**, *39*, 1321.
12. Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holý, A.; Schellekens, H.; De Clercq, E. *AIDS* **1991**, *5*, 21.
13. Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holý, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332.