SONOGASHIRA CROSS-COUPLING IN THE SYNTHESIS OF ACYCLIC NUCLEOSIDE PHOSPHONATES: PREPARATION OF 6-[(PHOSPHONOMETHOXY)ALKYNYL]- AND 6-[(PHOSPHONOMETHOXY)ALKYL]PYRIMIDINES

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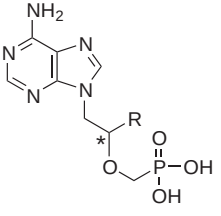
Several 6-[(phosphonomethoxy)alkyl]pyrimidines and 6-[(phosphonomethoxy)alkynyl]-pyrimidines were prepared as saturated and unsaturated carba-analogues of antivirally active 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine. As the key step of their synthesis the Sonogashira cross-coupling reaction was successfully applied. The replacement of the C–O moiety by the C–C bond resulted in the loss of biological activity.

Keywords: Nucleotide analogues; Acyclic nucleoside phosphonates; Pyrimidines; Sonogashira reaction; Cross-coupling reactions; Nucleosides; Alkynes; Hydrogenation; Antivirals.

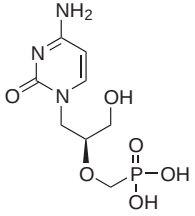
2-(Phosphonomethoxy)alkyl derivatives of purine and pyrimidine bases – acyclic nucleoside phosphonates (ANPs) – possess significant antiviral and cytostatic activity[1]. These nucleotide analogues contain the isopolar phosphonomethyl ether moiety instead of the phosphate ester group, which excludes their enzymatic dephosphorylation and/or circumvents problems with intracellular phosphorylation necessary for the first step of nucleoside activation. Among the ANPs, particularly 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir) and [(*R*)-2-(phosphonomethoxy)-propyl]adenine ((*R*)-PMPA, tenofovir) are active against DNA viruses and retroviruses.

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

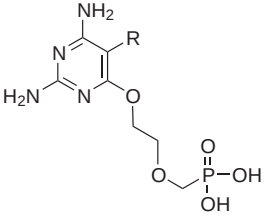
Recently we discovered a new type of antiviral ANPs originating from 2,4-diamino-6-hydroxypyrimidines[3–5]. These 6-[2-(phosphonomethoxy)ethoxy]-pyrimidine derivatives (PMEO-DAPy) significantly inhibit replication of retroviruses and herpes viruses in cell cultures. This novel subclass of pyrimidine ANPs can be considered as analogues of 2,6-diaminopurine with an open imidazole ring in the purine moiety.



PMEA R = H
(*R*)-PMPA R = (*R*)-CH_3



(*S*)-HPMPC



PMEO-DAPy R = H

Since the side chain attached via an ether bond at the 6-position of pyrimidine can be cleaved under alkaline condition[5], we decided to prepare more stable carba-analogues to study the influence of such substitution on the biological activity.

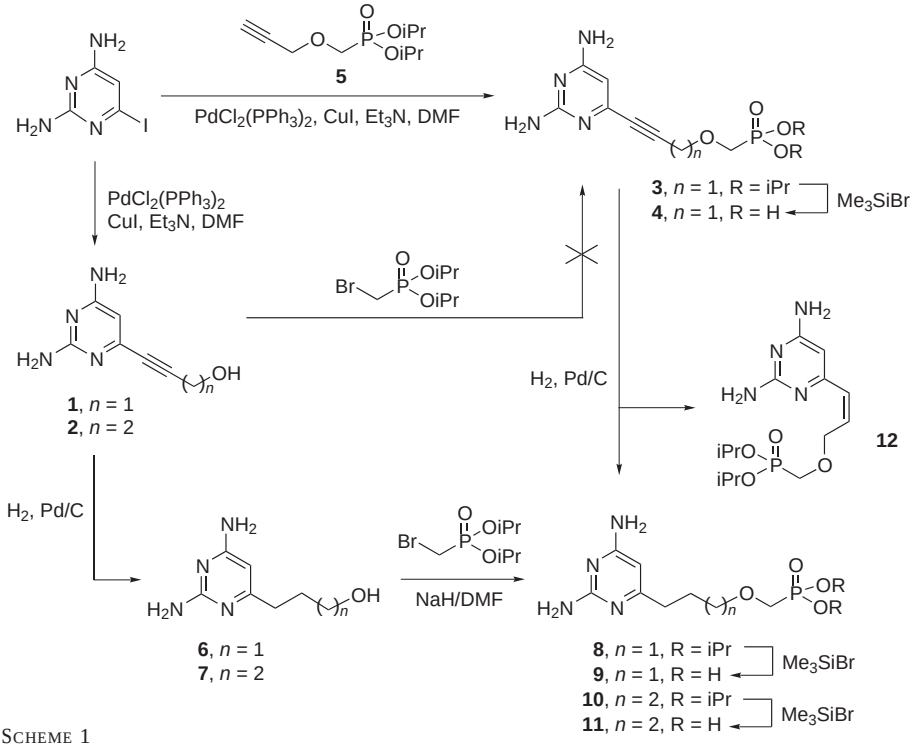
The Sonogashira cross-coupling reaction has been used in the synthesis of cytostatic 6-alkynylpurine nucleosides[6]. Recently we published a successful application of the Sonogashira cross-coupling reaction to the synthesis of cytostatic mono- and dialkynylpyrimidine derivatives[7]. Here, we report on an analogous synthetic approach which we selected for the formation of the C–C bond at the 6-position of pyrimidine ring in the synthesis of carba-analogues of PMEO-DAPy.

RESULTS AND DISCUSSION

The first approach consisted of the preparation of 2,4-diamino-6-(hydroxyalkynyl)pyrimidines[7] **1** and **2** via the Sonogashira cross-coupling reaction starting from 2,4-diamino-6-iodopyrimidine. Their subsequent alkylation with diisopropyl (bromomethyl)phosphonate was expected to afford the desired 2,4-diamino-6-{[(diisopropoxyphosphoryl)methoxy]alkynyl}pyrimidines, however, very complex mixtures were obtained under various standard conditions (NaH, Et_3N or *t*-BuONa as base, THF or DMF as solvent).

Another strategy was finally adopted to synthesise the designed 6-alkynyl phosphonate **3** (Scheme 1): The known[8] diisopropyl [(prop-2-yn-1-yloxy)-methyl]phosphonate (**5**) was prepared by the alkylation of propargyl alco-

hol with diisopropyl (bromomethyl)phosphonate in tetrahydrofuran using sodium hydride as a base. This alkyne was successfully coupled with 2,4-diamino-6-iodopyrimidine under standard Sonogashira conditions (Et_3N, CuI, $[PdCl_2(PPh_3)_2]$) in dimethylformamide at room temperature.



SCHEME 1

The resulting 6-alkynylpyrimidine **3** was hydrogenated over 5% palladium on charcoal in methanol, to give the diester of carba-analogue of PMEO-DAPy. With the very pure starting compound (purified by HPLC), the completely saturated derivative **8** was obtained in almost quantitative yield, while crude starting material (chromatographed on silica gel column only) afforded the partially hydrogenated compound **12** as the main product, perhaps due to poisoning of the catalyst.

On treatment with bromotrimethylsilane followed by hydrolysis, both diesters **3** and **8** smoothly provided the target phosphonic acids **4** and **9**. In contrast, the unstable diester **12** containing double bond in the side chain did not afford desired product either under the above reaction conditions or under the modified conditions (in the presence of 2,6-lutidine[9]); in both cases complex mixtures were obtained.

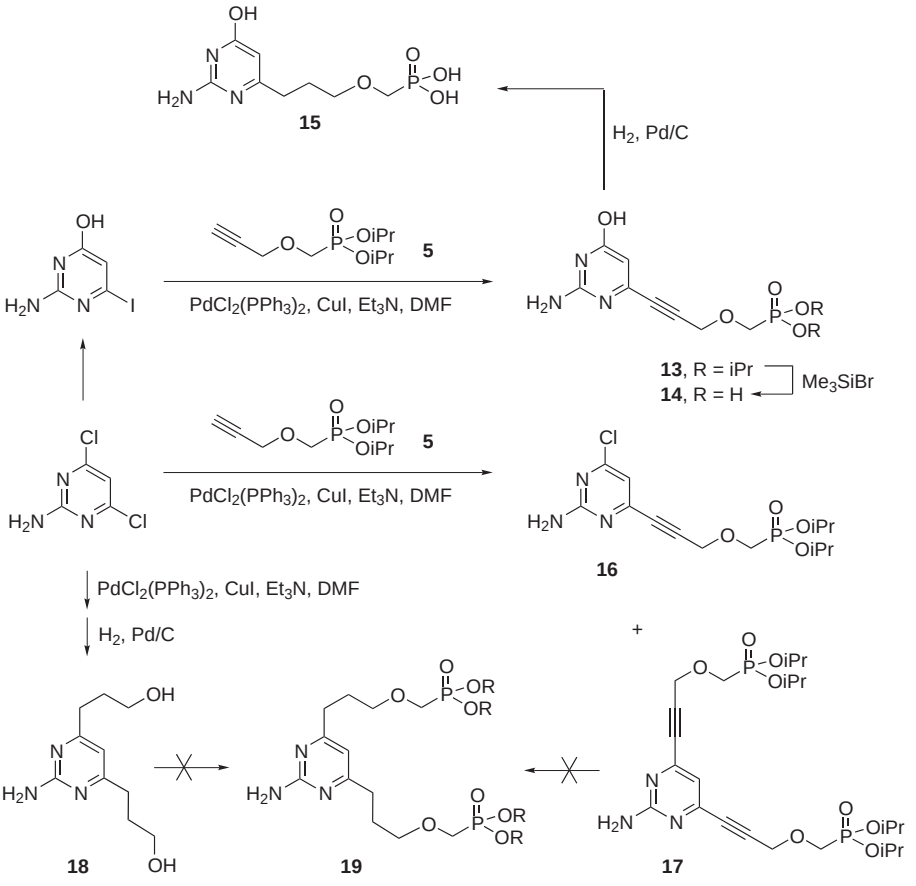
As an alternative approach to the preparation of saturated carba-analogue of PMEO-DAPy and its homologue **11** (Scheme 1), 2,4-diamino-6-(hydroxyalkynyl)pyrimidines[7] **1** and **2** were first catalytically hydrogenated on 5% palladium on charcoal to form hydroxy derivatives **6** and **7**, respectively, and then alkylated with diisopropyl (bromomethyl)phosphonate to give diesters **8** or **10**. While the homologous compound **10** was isolated from a complex reaction mixture in sufficient yield and smoothly transformed to the free phosphonic acid **11**, only traces of compound **8** were obtained. Thus the Sonogashira cross-coupling of 2,4-diamino-6-iodopyrimidine with preformed phosphonate **5** followed by catalytic hydrogenation seems to be the method of choice.

To extend the spectrum of carba-analogues and to study the influence of the substituent at the 4-position of pyrimidine, the above mentioned method was adjusted to prepare 4-hydroxy compounds **14** and **15** (Scheme 2). 2-Amino-4,6-dichloropyrimidine was converted to 2-amino-4-hydroxy-6-iodopyrimidine[10] by the procedure[11] using 55% hydroiodic acid and sodium iodide and then coupled with alkyne **5** to form diester **13**. Treatment with bromotrimethylsilane followed by hydrolysis afforded desired phosphonic acid **14**, which was again smoothly transformed to the saturated compound **15** by hydrogenation.

Since also 2-amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine[3] possesses interesting biological properties, we tried to prepare its carba-analogue **19**. Starting from 2-amino-4,6-dichloropyrimidine, the Sonogashira cross-coupling with alkyne **5** afforded always a mixture of mono- and dialkynyl compounds **16** and **17**. Even with excess of diisopropyl [(prop-2-yn-1-yloxy)methyl]phosphonate (**5**), the undesired monoalkynyl derivative was always the main product probably due to its limited subsequent reactivity in the Sonogashira cross-coupling reaction. Moreover, both phosphonates **16** and **17** were very unstable under standard reaction conditions used for the transformation of the diesters to free phosphonic acids[3,9]. To change the synthetic pathway, 2-amino-4,6-bis-(3-hydroxyprop-1-yn-1-yl)pyrimidine[6] prepared by the Sonogashira cross-coupling was easily hydrogenated in methanol over 5% palladium on charcoal to form bis(hydroxypropyl)pyrimidine **18**, but attempts at its dialkylation with diisopropyl (bromomethyl)phosphonate also completely failed.

In conclusion, the Sonogashira cross-coupling reaction is the method of choice for the C–C bond formation at position 6 of pyrimidine to form 2,4-disubstituted 6-{3-[(diisopropoxyphosphoryl)methoxy]prop-1-yn-1-yl}-pyrimidines as suitable starting materials for potentially biologically active

compounds. Using this methodology, a series of carba-analogues of biologically active PMEO-DAPy was prepared, containing either triple bond attached at the 6-position of pyrimidine (compounds **4** and **14**) or a completely saturated side chain (compounds **9**, **11** and **15**).



SCHEME 2

The target compounds were tested on their in vitro inhibition of the cell growth in mouse leukemia L1210 cells, human T-lymphoblastoid CCRF-CEM cell line, human promyelocytic leukemia HL-60 cells and human cervix carcinoma HeLa S3 cells. In contrast to active 6-alkynylpyrimidine derivatives **1**, **2** and 2-amino-4,6-bis(3-hydroxyprop-1-yn-1-yl)pyrimidine[7], none of the tested compounds (**4**, **6**, **7**, **9**, **11**, **14** and **15**) exhibited any significant activity.

Also in vitro effects against the DNA viruses and retroviruses were examined. Of the tested compounds, only carba-analogue **9** of the leading structure exhibited moderate anti-HIV activity (HIV-1: 0.15 μmol/ml; HIV-2: 0.05 μmol/ml). It is evident that the replacement of the C–O group at the 6-position of 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine[3] by the more stable C–C bond leads to the loss of antiviral activity.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried in vacuo over P_2O_5. Melting points were determined on a Büchi (Switzerland) melting point apparatus. NMR spectra were measured on an FT NMR spectrometer Varian UNITY 500 in dimethyl sulfoxide-d_6 or D_2O (^{1}H at 500 MHz and ^{13}C at 125.7 MHz frequency, chemical shifts given in ppm, coupling constants, *J*, in Hz). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionisation by Xe, accelerating voltage 8 kV, glycerol matrix). IR spectra were recorded on Nicolet 750 FT-IR (wavenumbers in cm^{-1}). Preparative HPLC were performed on Waters Delta 600. 2-Amino-4,6-dichloropyrimidine, propargyl alcohol, Me_3SiBr and $[PdCl_2(PPh_3)_2]$ were obtained from Sigma–Aldrich (Prague, Czech Republic). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å) in argon atmosphere.

2-Amino-4-hydroxy-6-iodopyrimidine[10]

A mixture of 2-amino-4,6-dichloropyrimidine (0.66 g, 4.0 mmol), sodium iodide (0.7 g, 4.7 mmol), 55% HI (7 ml) and several drops of acetone was stirred at room temperature for 2 days. Then solution of $NaHCO_3$ was slowly added (foam!) to alkaline pH and the resulting mixture was extracted with ethyl acetate. The organic phase was washed with saturated solution of sodium thiosulfate, dried over $MgSO_4$ and evaporated to give the required product (0.82 g, 86%). FABMS, *m/z* (%): 238 [MH^+] (50). ^{1}H NMR (DMSO-d_6): 11.20 br, 1 H (OH); 6.97 brs, 2 H (NH_2); 6.02 s, 1 H (H-5). ^{13}C NMR (DMSO-d_6): 161.13 and 154.58 (C-2 and C-4); 129.98 (C-6); 111.87 (C-5).

2,4-Diamino-6-{3-[(diisopropoxyphosphoryl)methoxy]prop-1-yn-1-yl}pyrimidine (**3**) and 2,4-Diamino-6-[3-(phosphonomethoxy)prop-1-yn-1-yl]pyrimidine (**4**)

Dimethylformamide (10 ml), diisopropyl [(prop-2-yn-1-yloxy)methyl]phosphonate (**5**; 1.0 g, 4.0 mmol) and Et_3N (0.3 ml, 2.15 mmol) were added through septum to an argon-purged flask containing 2,6-diamino-6-iodopyrimidine (470 mg, 2.0 mmol), CuI (20 mg, 0.1 mmol), $[PdCl_2(PPh_3)_2]$ (100 mg, 0.14 mmol) and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was coevaporated with toluene and ethanol. Crude product (350 mg, 51%) obtained by chromatography on a silica gel column (elution with 4–6% methanol in chloroform) was further purified by preparative HPLC (water–methanol). Yield 215 mg (31%) of **3**. FABMS, *m/z* (%): 343 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 6.45 brs, 2 H and 6.03 brs, 2 H (NH_2); 5.82 s, 1 H (H-5); 4.61 m, 2 H (P-OCH); 4.46 s, 2 H (OCH_2); 3.80 d, 2 H, *J*(P,CH) = 8.9 (P-CH_2); 1.26 d, 6 H and 1.25 d, 6 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 164.48 (C-4); 163.63 (C-2); 147.69 (C-6); 97.88

(C-5); 86.29 and 83.56 (C-1′ and C-2′); 70.55 d, 2 C, *J*(P,C) = 6.8 (P-OC); 63.51 d, *J*(P,C) = 166.0 (P-C); 59.76 d, *J*(P,C) = 15.6 (O-C); 23.99 d, 2 C, *J*(P,C) = 3.9 and 23.89 d, 2 C, *J*(P,C) = 4.9 (CH_3).

Compound **3** (170 mg, 0.5 mmol), acetonitrile (10 ml) and $BrSiMe_3$ (1.0 ml, 7.6 mmol) were stirred at room temperature overnight. After evaporation in vacuo and coevaporation 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 dissolved in hot water. Acetic acid was added to ca. pH 6 and the product precipitated as white solid (115 mg, 88%), m.p. 290 °C (decomp.). FABMS, *m/z* (%): 259 (MH^+) (60). IR (KBr): 3433, 3354, 3149, 2245, 1860, 1681, 1651, 1617, 1513, 1422, 1217, 1176, 1140, 1009, 941, 767, 593, 524, 455. ^{1}H NMR (D_2O + NaOD): 6.16 s, 1 H (H-5); 4.48 s, 2 H (O-CH_2); 3.62 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2). ^{13}C NMR (D_2O + NaOD): 164.77 (C-4); 162.61 (C-2); 147.87 (C-6); 99.85 (C-5); 87.72 and 83.65 (C-1′ and C-2′); 68.36 d, *J*(P,C) = 149.4 (P-C); 59.75 d, *J*(P,C) = 12.7 (O-CH_2). For $C_8H_{11}N_4O_4P \cdot 1/3H_2O$ (264.2) calculated: 36.37% C, 4.54% H, 21.21% N, 11.72% P; found: 36.53% C, 4.33% H, 20.92% N, 11.72% P.

Reduction of 2,4-Diamino-6-alkynylpyrimidines. General Procedure

2,4-Diamino-6-alkynylpyrimidine[7] **1** or **2** (5.0 mmol) was hydrogenated at atmospheric pressure in methanol (100 ml) over 5% palladium on charcoal (0.3 g) under stirring at room temperature for 20 h. The mixture was filtered through a pad of Celite, the catalyst was washed with hot water and hot methanol (100 ml each) and the filtrate was evaporated. Crystallisation (acetone–methanol) afforded required products **6** or **7**.

2,4-Diamino-6-(3-hydroxypropyl)pyrimidine (**6**). Yield 0.63 g (78%) of white solid, m.p. 160–162 °C. FABMS, *m/z* (%): 169 (MH^+) (100). IR (KBr): 3444, 3378, 3176, 2900, 2761, 1644, 1602, 1561, 1436, 1412, 1349, 1205, 1069, 1007, 926, 806, 762, 572, 446. ^{1}H NMR (DMSO-d_6): 6.12 brs, 2 H and 5.77 brs, 2 H (NH_2); 5.50 s, 1 H (H-5); 4.75 brs, 1 H (OH); 3.39 t, 2 H, *J*(3′,2′) = 7.6 (H-3′); 2.27 t, 2 H, *J*(1′,2′) = 6.5 (H-1′); 1.67 brpent, 2 H (H-2′). ^{13}C NMR (DMSO-d_6): 168.56 (C-4); 164.80 (C-2); 163.48 (C-6); 93.00 (C-5); 60.68 (C-3′); 33.78 (C-1′); 31.65 (C-2′). For $C_7H_{12}N_4O \cdot 1/6H_2O$ (171.2) calculated: 49.11% C, 7.26% H, 32.73% N; found: 49.12% C, 7.32% H, 32.49% N.

2,4-Diamino-6-(4-hydroxybutyl)pyrimidine (**7**). Yield 0.66 g (72%) of white solid, m.p. 226–228 °C. FABMS, *m/z* (%): 183 (MH^+) (100). IR (KBr): 3336, 3179, 2941, 1659, 1620, 1595, 1559, 1525, 1493, 1417, 1266, 1066, 999, 767, 573, 487. ^{1}H NMR (DMSO-d_6): 6.98 brs, 2 H and 6.53 brs, 2 H (NH_2); 5.69 s, 1 H (H-5); 3.39 t, 2 H, *J*(4′,3′) = 6.3 (H-4′); 2.35 t, 2 H, *J*(1′,2′) = 7.4 (H-1′); 1.56 brpent, 2 H and 1.41 brpent, 2 H (H-2′ and H-3′). ^{13}C NMR (DMSO-d_6): 165.03 (C-4); 162.93 (C-2); 159.94 (C-6); 93.56 (C-5); 60.53 (C-4′); 34.42 (C-1′); 32.07 (C-3′); 24.32 (C-2′). For $C_8H_{14}N_4O$ (182.2) calculated: 52.73% C, 7.74% H, 30.75% N; found: 52.55% C, 7.82% H, 30.58% N.

2,4-Diamino-6-{[3-(diisopropoxyphosphoryl)methoxy]propyl}pyrimidine (**8**) and
2,4-Diamino-6-{[3-(diisopropoxyphosphoryl)methoxy]prop-1-en-1-yl}pyrimidine (**12**)

Method A: 2,4-Diamino-6-{[3-(diisopropoxyphosphoryl)methoxy]prop-1-yn-1-yl}pyrimidine (**3**; 0.38 g, 1.1 mmol, purified by HPLC) was hydrogenated at atmospheric pressure in methanol (20 ml) over 5% palladium on charcoal (0.1 g) at room temperature under stirring for 2 days. The mixture was filtered through a pad of Celite, the catalyst was washed with hot methanol and the filtrate was evaporated to obtain crude product **8** (0.36 g, 94%), which was di-

rectly used in subsequent reaction. FABMS, *m/z* (%): 347 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 6.31 brs, 2 H and 5.92 brs, 2 H (NH_2); 5.57 s, 1 H (H-5); 4.60 m, 2 H (P-OCH); 3.69 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.49 t, 2 H, *J*(3′,2′) = 6.5 (H-3′); 2.30 t, 2 H, *J*(1′,2′) = 7.6 (H-1′); 1.77 brpent, 2 H (H-2′); 1.25 d, 6 H and 1.24 d, 6 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 166.17 (C-4); 164.86 (C-2); 162.73 (C-6); 93.21 (C-5); 72.18 d, *J* = 11.7 (C-3′); 70.26 d, 2 C, *J*(P,C) = 6.4 (P-OC); 64.84 d, *J*(P,C) = 164.55 (P-C); 33.02 (C-1′); 28.00 (C-2′); 24.03 d, 2 C, *J*(P,C) = 3.9 and 23.93 d, 2 C, *J*(P,C) = 4.9 (CH_3).

When crude starting compound **3** (without HPLC purification, 0.34 g, 1.0 mmol) was used, 2,4-diamino-6-{[3-(diisopropoxyphosphoryl)methoxy]prop-1-en-1-yl}pyrimidine (**12**) was obtained as the only product (140 mg, 42%). FABMS, *m/z* (%): 345 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 6.28 brs, 2 H and 5.80 brs, 2 H (NH_2); 6.06 dt, 1 H, *J*(1′,3′) = 2.0, *J*(1′,2′) = 12.0 (H-1′); 5.79 dt, 1 H, *J*(2′,3′) = 5.4, *J*(1′,2′) = 12.0 (H-2′); 5.62 s, 1 H (H-5); 4.69 t, 2 H, *J*(3′,1′) = 2.0, *J*(3′,2′) = 5.4 (H-3′); 4.60 m, 2 H (P-OCH); 3.74 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 1.24 d, 6 H and 1.23 d, 6 H, *J*(CH_3,CH) = 6.2 (CH_3). ^{13}C NMR (DMSO-d_6): 165.10 (C-4); 163.19 (C-2); 161.10 (C-6); 134.20 (C-1′); 129.09 (C-2′); 95.31 (C-5); 70.76 d, *J* = 11.7 (C-3′); 70.30 d, 2 C, *J*(P,C) = 6.3 (P-OC); 64.41 d, *J*(P,C) = 165.5 (P-C); 24.02 d, 2 C, *J*(P,C) = 3.9 and 23.91 d, 2 C, *J*(P,C) = 4.4 (CH_3).

Method B: 2,4-Diamino-6-(3-hydroxypropyl)pyrimidine (**6**; 0.12 g, 0.7 mmol) and NaH (30 mg, 0.8 mmol, 60% dispersion in mineral oil) were stirred in DMF (4 ml) at 60 °C for 1.5 h. Then diisopropyl (bromomethyl)phosphonate (0.33 g, 1.2 mmol) was added and the heating was continued for 25 h. The solvent was evaporated in vacuo and the residue was coevaporated with toluene and ethanol. Chromatography on a silica gel column afforded only traces of product **8** identical with that obtained by method *A*.

2,4-Diamino-6-[3-(phosphonomethoxy)propyl]pyrimidine (**9**)

Compound **8** (350 mg, 1.0 mmol), acetonitrile (10 ml) and $BrSiMe_3$ (1.0 ml, 7.6 mmol) were stirred at room temperature overnight. After evaporation in vacuo and codistillation with acetonitrile, the residue was treated with water and aqueous ammonia was added to alkaline reaction. The mixture was evaporated to dryness and the residue was dissolved in hot water. Acetic acid was added to ca. pH 6 and, after addition of methanol, the product precipitated as white solid (140 mg, 53%), m.p. 267–269 °C (decomp.). FABMS, *m/z* (%): 263 [MH^+] (20). IR (KBr): 3378, 3279, 3038, 2987, 2479, 2375, 1857, 1669, 1638, 1522, 1429, 1165, 1044, 924, 536, 457. ^{1}H NMR (D_2O + NaOD): 5.94 s, 1 H (H-5); 3.58 t, 2 H, *J*(3′,2′) = 6.7 (H-3′); 3.49 d, 2 H, *J*(P,CH) = 8.7 (P-CH_2); 2.46 t, 2 H, *J*(1′,2′) = 7.6 (H-1′); 1.87 brpent, 2 H (H-2′). ^{13}C NMR (D_2O + NaOD): 169.67 (C-4); 164.93 (C-2); 162.51 (C-6); 94.88 (C-5); 71.80 d, *J* = 10.8 (C-3′); 68.64 d, *J*(P,C) = 150.4 (P-C); 32.67 (C-1′); 27.51 (C-2′). For $C_8H_{15}N_4O_4P \cdot 1/4H_2O$ (266.7) calculated: 36.03% C, 5.86% H, 21.01% N, 11.61% P; found: 36.32% C, 5.86% H, 20.79% N, 11.49% P.

2,4-Diamino-6-[4-(phosphonomethoxy)butyl]pyrimidine (**11**)

2,4-Diamino-6-(3-hydroxybutyl)pyrimidine (**7**; 550 mg, 3.0 mmol) and NaH (150 mg, 3.9 mmol, 60% dispersion in mineral oil) were stirred in DMF (10 ml) at 60 °C for 1.5 h, then diisopropyl (bromomethyl)phosphonate (1.48 g, 5.7 mmol) was added and the heating was continued for 24 h. The solvent was evaporated in vacuo and the residue was coevaporated with toluene and ethanol. Water was added and the mixture was extracted with ethyl acetate. Chromatography on a silica gel column (methanol–ethyl acetate) afforded

crude intermediate **10** (2,4-diamino-6-{[3-(diisopropoxyphosphoryl)methoxy]butyl}pyrimidine): 280 mg (26%). FABMS, *m/z* (%): 361 [MH^+] (100).

Compound **10** (280 mg, 0.8 mmol, codistilled with acetonitrile), acetonitrile (20 ml) and $BrSiMe_3$ (1.0 ml, 7.6 mmol) were stirred at room temperature overnight. After evaporation in vacuo and codistillation with acetonitrile, the residue was treated with water and aqueous ammonia was added to alkaline reaction. The mixture was evaporated to dryness, and the residue dissolved in water was applied onto a column of Dowex 50X8 (H^+-form, 15 ml) and washed with water. Elution with 2.5% aqueous ammonia and evaporation in vacuo afforded crude product as ammonium salt. This residue dissolved in minimum volume of water was applied onto a Dowex 1X2 (acetate, 15 ml) column, which was then washed with water followed by gradient of acetic acid (0–2 mol l^{-1}, 0.5 l each) and hot formic acid (2 mol l^{-1}; the product was very insoluble). Combined fractions containing product were evaporated, the residue was three times codistilled with water and crystallised from water to afford product **11** as yellowish solid (90 mg, 41%), m.p. 200–202 °C (decomp.). FABMS, *m/z* (%): 277 [MH^+] (100). IR (KBr): 3383, 3204, 2926, 1658, 1643, 1518, 1406, 1140, 1116, 933, 769, 557. ^{1}H NMR (D_2O + NaOD): 5.92 s, 1 H (H-5); 3.58 t, 2 H, *J*(3′,2′) = 7.0 (H-4′); 3.48 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 2.42 t, 2 H, *J*(1′,2′) = 7.5 (H-1′); 1.61 m, 4 H (H-2′ and H-3′). For $C_9H_{17}N_4O_4P$ (276.2) calculated: 39.13% C, 6.20% H, 20.28% N; found: 38.92% C, 6.34% H, 19.95% N.

2-Amino-4-hydroxy-6-{[3-(diisopropoxyphosphoryl)methoxy]prop-1-yn-1-yl}pyrimidine (**13**) and 2-Amino-4-hydroxy-6-[3-(phosphonomethoxy)prop-1-yn-1-yl]pyrimidine (**14**)

Dimethylformamide (15 ml), diisopropyl [(prop-2-yn-1-yloxy)methyl]phosphonate (**5**; 1.0 g, 4 mmol) and Et_3N (0.3 ml, 2.15 mmol) were added through septum to an argon-purged flask containing 2-amino-4-hydroxy-6-iodopyrimidine (500 mg, 2.1 mmol), CuI (20 mg, 0.1 mmol) and [$PdCl_2(PPh_3)_2$] (160 mg, 0.2 mmol), and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was coevaporated with toluene and ethanol. Crude product obtained by chromatography on a silica gel column (ethyl acetate–methanol) was further purified by preparative HPLC (water–methanol). Yield of intermediate **13** was 410 mg (57%) of yellowish solid. FABMS, *m/z* (%): 344 [MH^+] (10). ^{1}H NMR (DMSO-d_6): 10.98 brs, 1 H (OH); 6.67 brs, 2 H (NH_2); 5.69 s, 1 H (H-5); 4.61 m, 2 H (P-OCH); 4.46 s, 2 H (O-CH_2); 3.79 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 1.25 d, 6 H and 1.24 d, 6 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 162.28 (C-4); 156.31 (C-2); 148.41 (C-6); 105.71 (C-5); 86.30 and 85.57 (C-1′ and C-2′); 70.58 d, 2 C, *J*(P,C) = 6.4 (P-OC); 63.62 d, *J*(P,C) = 165.8 (P-C); 59.75 d, *J* = 13.5 (C-3′); 23. 99 d, 2 C and 23.90 d, 2 C, *J*(P,C) = 4.4 (CH_3).

Compound **13** (320 mg, 0.93 mmol), acetonitrile (20 ml) and $BrSiMe_3$ (1.2 ml, 9.1 mmol) were stirred at room temperature overnight. After evaporation in vacuo and coevaporation with acetonitrile, the residue was treated with water and aqueous ammonia was added to alkaline reaction. The mixture was evaporated to dryness, and the residue dissolved in water was applied onto a column of Dowex 50X8 (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 dissolved in minimum volume of water was applied onto a Dowex 1X2 (acetate, 15 ml) column, which was then washed with water followed by gradient of acetic acid (0–2 mol l^{-1}, 0.5 l each) and hot formic acid (4 mol l^{-1}; the product was very insoluble). Combined fractions containing product were evaporated, the residue was three times coevaporated with water and crystallised from water–ethanol to afford product **14** as white

solid (175 mg, 73%), m.p. 250 °C (decomp.). FABMS, *m/z* (%): 260 (MH^+) (10). IR (KBr): 3396, 3193, 2927, 2788, 2242, 1869, 1728, 1654, 1483, 1379, 1216, 1134, 1085, 944, 922, 826, 540. ^{1}H NMR (D_2O + NaOD): 6.00 s, 1 H (H-5); 4.47 s, 2 H (O-CH_2); 3.61 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2). ^{13}C NMR (D_2O + NaOD): 173.39 (C-4); 161.31 (C-2); 144.68 (C-6); 103.21 (C-5); 84.22 and 81.43 (C-1′ and C-2′); 65.57 d, *J*(P,C) = 149.6 (P-C); 57.21 d, *J*(P,C) = 12.7 (O-CH_2). For $C_8H_{10}N_3O_5P \cdot H_2O$ (277.2) calculated: 34.67% C, 4.36% H, 15.16% N, 11.17% P; found: 34.55% C, 4.11% H, 14.84% N, 11.12% P.

2-Amino-4-hydroxy-6-[3-(phosphonomethoxy)propyl]pyrimidine (**15**)

2-Amino-4-hydroxy-6-[3-(phosphonomethoxy)prop-1-yn-1-yl]pyrimidine (**14**; 125 mg, 0.48 mmol) was hydrogenated at atmospheric pressure in water (40 ml; several drops of Et_3N added to dissolve starting compound) over 5% palladium on charcoal (0.1 g) under stirring at room temperature for 7 h. The mixture was filtered through a pad of Celite, the catalyst was washed with hot water/Et_3N and the filtrate was evaporated to dryness. This residue dissolved in minimum volume of water was applied onto a Dowex 1X2 (acetate, 10 ml) column, which was then washed with water followed by gradient of acetic acid (0–0.3 mol l^{-1}). Combined fractions containing the product were concentrated and white solid **15** (70 mg, 55%) was filtered off, m.p. 246–247 °C (decomp.). FABMS, *m/z* (%): 264 [MH^+] (70). IR (KBr): 3394, 3287, 3192, 2946, 2889, 1702, 1686, 1664, 1547, 1485, 1236, 1204, 1163, 1033, 915, 832, 529. ^{1}H NMR (D_2O + NaOD): 5.72 s, 1 H (H-5); 3.59 t, 2 H, *J*(3′,2′) = 6.8 (H-3′); 3.48 d, 2 H, *J*(P,CH) = 8.7 (P-CH_2); 2.42 t, 2 H, *J*(1′,2′) = 7.8 (H-1′); 1.88 m, 2 H (H-2′). ^{13}C NMR (D_2O + NaOD): 176.67 (C-4); 168.99 (C-2); 163.83 (C-6); 100.15 (C-5); 72.11 d, *J* = 10.3 (C-3′); 68.73 d, *J*(P,C) = 149.9 (P-C); 32.53 (C-1′); 27.69 (C-2′). For $C_8H_{13}N_3O_5P \cdot H_2O$ (281.2) calculated: 34.17% C, 5.73% H, 14.94% N, 11.01% P; found: 36.07% C, 5.80% H, 14.84% N, 10.86% P.

2-Amino-4-chloro-6-{[3-(diisopropoxyphosphoryl)methoxy]prop-1-yn-1-yl}pyrimidine (**16**) and 2-Amino-4,6-bis{[3-(diisopropoxyphosphoryl)methoxy]prop-1-yn-1-yl}pyrimidine (**17**)

Dimethylformamide (15 ml), diisopropyl [(prop-2-yn-1-yloxy)methyl]phosphonate (**5**; 1.4 g, 6.0 mmol) and Et_3N (0.6 ml, 4.3 mmol) were added through septum to an argon-purged flask containing 2-amino-4,6-dichloropyrimidine (330 mg, 2.0 mmol), CuI (30 mg, 0.15 mmol) and [$PdCl_2(PPh_3)_2$] (400 mg, 0.5 mmol), and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was coevaporated with toluene and ethanol. Crude product obtained by chromatography on a silica gel column (ethyl acetate–methanol) was further purified by preparative HPLC (water–methanol) to obtain pure monoalkynyl derivative **16** and dialkynyl derivative **17**.

Yield of **16**: 320 mg (44%) of white solid. FABMS, *m/z* (%): 362 [MH^+] (60). ^{1}H NMR (DMSO-d_6): 7.31 brs, 2 H (NH_2); 6.82 s, 1 H (H-5); 4.61 dsept, 2 H, *J*(CH,CH_3) = 7.1, *J*(P,OCH) = 7.7 (P-OCH); 4.53 s, 2 H (O-CH_2); 3.83 d, 2 H, *J*(P,CH) = 8.9 (P-CH_2); 1.26 d, 6 H and 1.25 d, 6 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 163.44 (C-2); 160.77 (C-4); 151.54 (C-6); 110.95 (C-5); 88.25 and 84.17 (C-1′ and C-2′); 70.55 d, 2 C, *J*(P,C) = 6.4 (P-OC); 63.78 d, *J*(P,C) = 166.0 (P-C); 59.64 d, *J* = 15.1 (C-3′); 23.96 d, 2 C, *J*(P,C) = 3.9 and 23.87 d, 2 C, *J*(P,C) = 4.4 (CH_3).

Yield of **17**: 210 mg (19%) of white solid. FABMS, *m/z* (%): 560 [MH^+] (30). ^{1}H NMR (DMSO-d_6): 7.02 brs, 2 H (NH_2); 6.80 s, 1 H (H-5); 4.61 m, 4 H (P-OCH); 4.53 s, 4 H (O-CH_2); 3.83 d, 4 H, *J*(P,CH) = 8.9 (P-CH_2); 1.26 d, 12 H and 1.25 d, 12 H, *J*(CH_3,CH) = 6.1

(CH_3). ^{13}C NMR (DMSO-d_6): 163.72 (C-2); 150.69, 2 C (C-4 and C-6); 113.89 (C-5); 87.80, 2 C and 84.61, 2 C (C-1′, C-1″, C-2′ and C-2″); 70.53 d, 4 C, J(P,C) = 6.4 (P-OC); 63.73 d, 2 C, J(P,C) = 166.0 (P-C); 59.67 d, 2 C, J = 15.1 (C-3′ and C-3″); 23.92 d, 4 C, J(P,C) = 3.9 and 23.86 d, 4 C, J(P,C) = 4.4 (CH_3).

2-Amino-4,6-bis(3-hydroxypropyl)pyrimidine (**18**)

2-Amino-4,6-bis(3-hydroxyprop-1-yn-1-yl)pyrimidine (340 mg, 1.7 mmol) was hydrogenated at atmospheric pressure in methanol (70 ml) over 5% palladium on charcoal (0,3 g) under stirring at room temperature for 20 h. The mixture was filtered through a pad of Celite, the catalyst was washed with hot methanol and the filtrate was evaporated. Preparative TLC (20% methanol–chloroform) followed by crystallisation (acetone–methanol) afforded desired product. Yield 170 mg (47%), m.p. 96–97 °C. FABMS, *m/z* (%): 212 (MH^+) (100). IR (KBr): 3370, 3326, 3176, 2921, 2875, 1654, 1587, 1564, 1471, 1410, 1372, 1351, 1226, 1066, 1052, 916, 711, 552, 489. ^1H NMR (DMSO-d_6): 6.32 s, 1 H (H-5); 6.34 brs, 2 H (NH_2); 4.50 brs, 2 H (OH); 3.40 t, 4 H, J(3′,2′) = 6.5 (H-3′ and H-3″); 2.45 t, 4 H, J(1′,2′) = 7.6 (H-1′ and H-1″); 1.73 brpent, 4 H (H-2′ and H-2″). ^{13}C NMR (DMSO-d_6): 163. 36 (C-2); 117.36, 2 C (C-2 and C-6); 107.81 (C-5); 60.47, 2 C (C-3′ and C-3″); 33.72 (C-1′ and C-1″); 31.67, 2 C (C-2′ and C-2″). For $C_{10}H_{17}N_3O_2$ (211.3) calculated: 58.85% C, 8.11% H, 19.89% N; found: 56.61% C, 8.25% H, 19.58% N.

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REFERENCES

1. Reviews: a) Holý A. in: *Advances in Antiviral Drug Design* (E. De Clercq, Ed.), p. 179. JAI Press, Greenwich (CT) 1994; b) Holý A. in: *Recent Advances in Nucleosides: Chemistry and Chemotherapy* (C. K. Chu, Ed.), p. 167. Elsevier Science B.V., Amsterdam 2002; c) Holý A.: *Curr. Pharm. Des.* **2003**, 2567.
2. For reviews, see: a) De Clercq E.: *Collect. Czech. Chem. Commun.* **1998**, *63*, 480; b) Snoeck R., Noel J. C., Muller C., De Clercq E., Bossens M.: *J. Med. Virol.* **2000**, *60*, 205.
3. 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; c) Balzarini J., Pannecouque C., Naesens J., Andrei G., Snoeck R., De Clercq E., Hocková D., Holý A.: *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 1321.
4. Hocková D., Holý A., Masojídková M., Andrei G., Snoeck R., De Clercq E., Balzarini J.: *J. Med. Chem.* **2003**, *46*, 5064.

5. Hocková D., Holý A., Masojídková M., Andrei G., Snoeck R., De Clercq E., Balzarini J.: *Bioorg. Med. Chem.* **2004**, *12*, 3197.
6. Nauš P., Votruba I., Hocek M.: *Collect. Czech. Chem. Commun.* **2004**, *69*, 1955.
7. Hocková D., Holý A., Masojídková M., Votruba I.: *Tetrahedron* **2004**, *60*, 4983.
8. Pudovik A. N., Khusainova N. G.: *Zh. Obshch. Khim.* **1968**, *38*, 678.
9. Alexander P., Krishnamurthy V. V., Prisbe E. J.: *J. Med. Chem.* **1996**, *39*, 1321.
10. Hirayama T., Kamada M., Mimura M., Tsurumi H.: *Chem. Pharm. Bull.* **1976**, *24*, 507.
11. Gomtsyan A., Didomenico S., Lee C.-H., Matulenko M. A., Kim K., Kowaluk E. A., Wismer C. T., Mikusa J., Yu H., Kohlhaas K., Jarvis M. F., Bhagwat S. S.: *J. Med. Chem.* **2002**, *45*, 3639.