# SYNTHESIS OF QUATERNARY 1-[2-(PHOSPHONOMETHOXY)ETHYL] DERIVATIVES OF 2,4-DIAMINOPYRIMIDINE AND RELATED ACYCLIC NUCLEOTIDE ANALOGS

Antonín HOLÝ[a1,*], Miloš BUDĚŠÍNSKÝ[a2], Jaroslav PODLAHA[b1] and Ivana CÍSAŘOVÁ[b2]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] uochb@uochb.cas.cz, [2] budesinsky@uochb.cas.cz*

[b] *Department of Inorganic Chemistry, Charles University, 128 40 Prague 2, Czech Republic; e-mail: [1] podlaha@prfdec.natur.cuni.cz, [2] cisarova@prfdec.natur.cuni.cz*

Received December 15, 1998
Accepted January 12, 1999

*Dedicated to the memory of Dr Miroslav Protiva.*

Quaternization of 2,4-diaminopyrimidine (**2**) by diisopropyl 2-chloroethoxymethanephosphonate (**3**) followed by bromotrimethylsilane treatment and subsequent hydrolysis gave zwitterionic *N*1-[2-(phosphonomethoxy)ethyl] derivative, hydrogen {[2-(2,4-diaminopyrimidin-1-io)ethoxy]methyl}phosphonate (**5**). Its structure was confirmed by X-ray crystallography. The same product was obtained from 2-amino-4-[(dimethylaminomethylene)amino]pyrimidine (**6**) by an analogous reaction sequence followed by an aqueous ammonia treatment after the transsilylation reaction. Also the quaternizations of 4,6-diaminopyrimidine (**7**) and 2,4,6-triaminopyrimidine (**8**) with the halo derivative **3** afforded the zwitterionic *N*1-substituted compounds **9** and **10**, respectively. In contrast to this regiospecific reaction, 2-aminopyrimidine (**11**) gave on treatment with compound **3** and following deprotection the *exo*-*N*2-isomer **13**. This compound was also obtained by the reaction starting from 2-[(dimethylaminomethylene)amino]pyrimidine (**12**) which was prepared by treatment of compound **11** with dimethylformamide dineopentyl acetal. Also 2,3-diaminopyridine (**14**) gave by the above reaction a mixture of 2-amino-3-{[2-(phosphonomethoxy)ethyl]amino}pyridine (**15**) and quaternary *N*1-[2-(phosphonomethoxy)ethyl] derivative (**16**). None of these analogs of the antiviral PMEDAP exhibited any antiviral activity against DNA viruses or retroviruses, nor any cytostatic activity.

**Key words**: Acyclic nucleotide analogs; Pyrimidines; Phosphonates; Antivirals; PMEDAP; NMR spectroscopy, $^{1}H$ and $^{13}C$; Crystal structure.

9-[2-(Phosphonomethoxy)ethyl]-2,6-diaminopurine (**1**, PMEDAP) is a powerful antiviral drug active both against DNA viruses[1] and retroviruses[2] (HIV); *in vivo* treatment of mice infected with either Moloney murine sar-

coma virus (MSV), Friend leukemia virus (FLV), or murine cytomegalovirus (MCMV) revealed the high therapeutic effect of this compound[3,4]. Also its high activity against hepatitis B virus (HBV) has been firmly established[5]. It was recently shown that PMEDAP also has a significant cytostatic activity[6]. Modifications of this lead structure which concentrated mainly on the substitution of its *N*6-amino function, resulted in numerous biologically active compounds with antiviral[7,8] and cytostatic[9] properties.

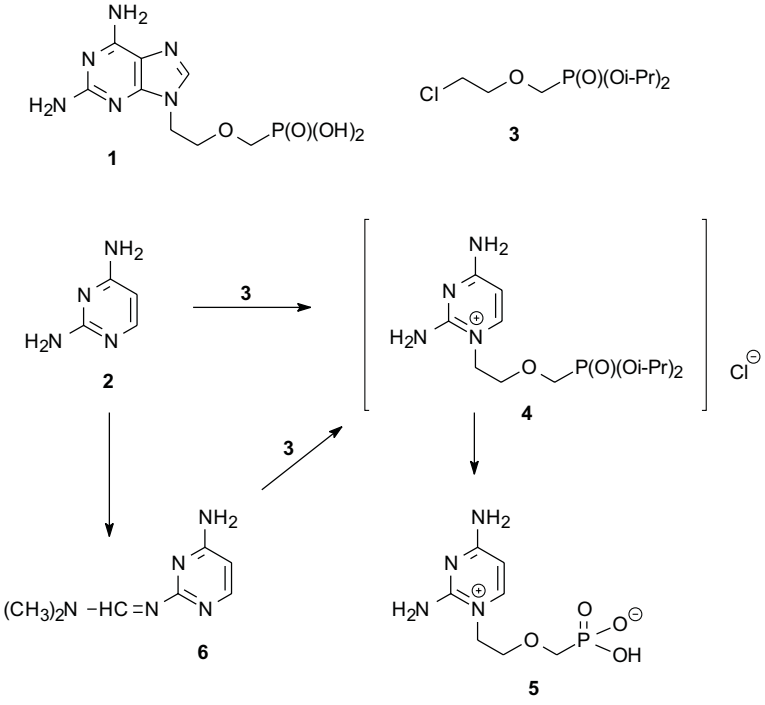
The most conspicuous structural feature of the purine system in the PMEDAP molecule is its pyrimidine part bearing two amino functions. 2-Aminopurine substituted at the position 6 was postulated as a pharmacophor of the acyclic nucleoside phosphonates active against MSV (ref.[10]). Thus, it seemed of interest to synthesize and investigate compounds bearing the 2-(phosphonomethoxy)ethyl (PME) chain in 2-aminopyrimidine or 2,4-diaminopyrimidine base, or their deaza analogs.

The synthesis of pyrimidine and/or purine acyclic nucleoside phosphonates from the heterocyclic bases containing acid NH function is mainly based on an *N*-alkylation of the base with an alkylation agent bearing the structural features of the PME chain and protected at the phosphonate residue by ester functions[11]. Diethyl esters of [2-(tosyloxy)ethoxy]methylphosphonic acid[12] and/or [(2-chloroethoxy)methyl]phosphonic acid were originally utilized for this purpose[13]. However, this approach was considerably improved by the use of the diisopropyl ester **3** (ref.[10]) which eliminates the formation of *N*-ethyl derivatives as side products of the *N*-alkylation.

All these alkylations take place in the presence of a base: the heterocyclic purine or pyrimidine bases are transformed to their sodium salts by the reaction with NaH or, the alkylation is performed in the presence of cesium carbonate[13] or, 1,7-diazabicyclo[5.4.0]undec-7-ene[11] (DBU). However, in 2,4-diaminopyrimidine and related compounds of pyrimidine and pyridine series lacking NH-groupings, the key-step in the reaction sequence for the synthesis of the desired phosphonate analogs is a quaternization reaction. Such a reaction with compound **3** was also applied to the quaternization of nicotinamide[14].

Reaction of 2,4-diaminopyrimidine (**2**) with excess compound **3** used as a solvent takes place at moderately increased temperature. It affords an ionic product bearing a pronounced electropositive charge as witnessed by its electrophoretic mobility at slightly alkaline pH. This intermediate was not further characterized; after removal of excess compound **3** it was directly submitted to transsilylation with $BrSiMe_3$ followed by hydrolysis. The reaction mixture contained, in addition to unreacted **2**, a single product with

slightly acidic character (Scheme 1). It was deionized by Dowex 50 chromatography and afforded compound **5** by further purification on Dowex 1 X 2. No other regioisomer of compound **5** was detected in the reaction mixture[15]. The assignment of this structure is based on $^1H$ and $^{13}C$ spectra (*vide infra*). Also the UV spectrum ($\lambda_{max}$ 270 nm) of the product cor-




SCHEME 1

responds rather to the value published for the quaternary *N*1-methyl derivative ($\lambda_{max}$ 271 nm) than to the UV spectrum of the isomeric 4-amino-2-methylaminopyrimidine[16] ($\lambda_{max}$ 276 nm). An identical product **5** was also obtained from the formamidine **6** which was prepared from 2,4-diaminopyrimidine (**2**) by the action of dimethylformamide dineopentyl acetal[17] or dimethyl acetal. The amidine formation proceeds solely at the 2-$NH_2$ function even when the reaction is performed at elevated temperature; the *N*4-mono- and *N*2,*N*4-bis(amidine)-protected derivatives were detected as impurities in the crude reaction product only in spite of a careful workup[18] (as shown by NMR analysis of the crude **6**).

Clearly these data cannot unequivocally distinguish between the zwitterionic quaternary structure **5a**, zwitterionic structure with protonated (2- or

4-) immonium function (**5b**) or the phosphonic acid structure **5c**. However, the electrophoretic mobility which is much lower than that expected for the monoanion derived from cytosine or 2,6-diaminopurine acyclic phosphonate, speaks in favor of the quaternary structure, *i.e.* hydrogen 2-{[2-(2,4-diaminopyrimidin-1-io)ethoxy]methyl}phosphonate (**5a**).

The final structural proof of compound **5** was provided by low-temperature X-ray structure determination. All hydrogen atoms were unambiguously located and refined. The structure is zwitterionic with singly deprotonated phosphonate group (Fig. 1). The bond lengths in the pyrimidine ring, which is planar within ±0.017 Å including N2 and N4 atoms, indicate certain degree of electron delocalization over all its nitrogen atoms. While the geometry around N1 is typical of a quaternary *N*-alkylpyridinium derivative, the distances to the exocyclic amino groups are somewhat shortened compared to simple aromatic amines; more significantly, the $NH_2$ hydrogens lie almost exactly within the heteroaromatic plane (less so for N4 but even these N3–C–N–H torsion angles are below 9(1)° ). On the other hand, the electron delocalization in the C4 to C6 part is incomplete and, although the hybridization at C5 and C6 is strictly $sp^2$, the C4–C5 bond is by 0.088(2) Å longer than C5–C6. The overall geometry of the pyrimidine base is almost identical with that of bis(2,4-diaminopyrimidinium) sulfate monohydrate[19], the only closely related structure found in Cambridge Structural Database. Though this compound is therein formulated[19] with the charge localized at N1, such a presentation is purely formal: no direct evidence of charge localization can be obtained from a standard electron density determination.

The conformation of the side chain is obviously dictated by intermolecular hydrogen bonding. The phosphonate groups are linked by a

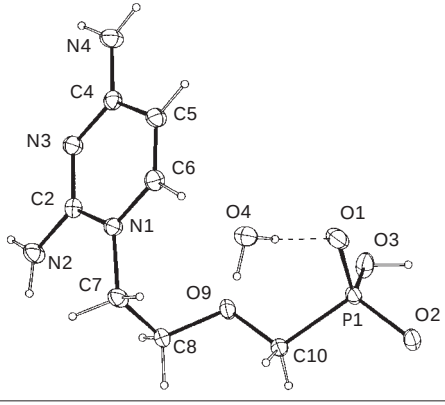
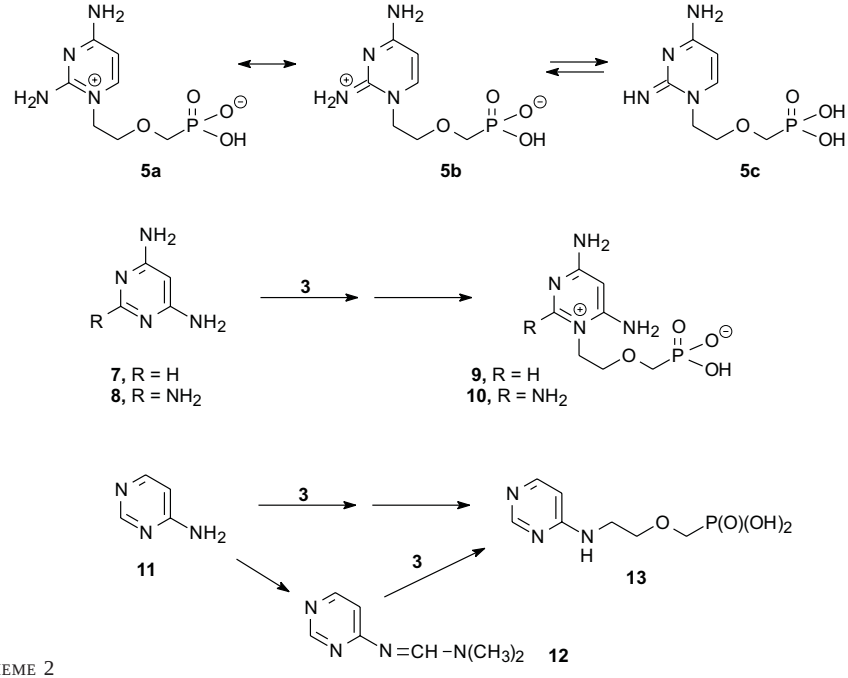



FIG. 1
Perspective view of $5 \cdot H_2O$ with atom labelling (50% probability ellipsoids)

short, centrosymmetric double hydrogen bond (O2···O3 2.538 Å) supported by longer $NH_2$–phosphonate and $NH_2$–N3 interactions, either direct or mediated by the water molecules. In this bonding, all the amino and water hydrogens participate as donors and the phosphonate and water oxygens and N3 as acceptors. The ether oxygen O9 and the heterocyclic nitrogen N1 are not involved in hydrogen bonding. In the crystal, the zwitterions are linked into two-dimensional layers propagating approximately parallel to the crystallographic *ac*-plane which is also the direction of the preferential growth of the crystals.

Similarly, 4,6-diaminopyrimidine (**7**) and 2,4,6-triaminopyrimidine (**8**) gave by the reaction with the phosphonate **3** quaternary *N*1-[2-(phosphonomethoxy)ethyl] derivatives **9** and **10**, respectively (Scheme 2), without any detectable formation of other regioisomers. Their structure was also assigned on the basis of NMR data (*vide infra*) and corroborated by comparing their UV spectra with those of corresponding methyl derivatives: the unequivocal course of the reaction corresponds to a similar reaction of the base **7** with methyl iodide which affords the quaternary *N*1-methyl derivative as the only reaction product[20]. Its UV characteristics ($\lambda_{max}$ 265 nm)




SCHEME 2

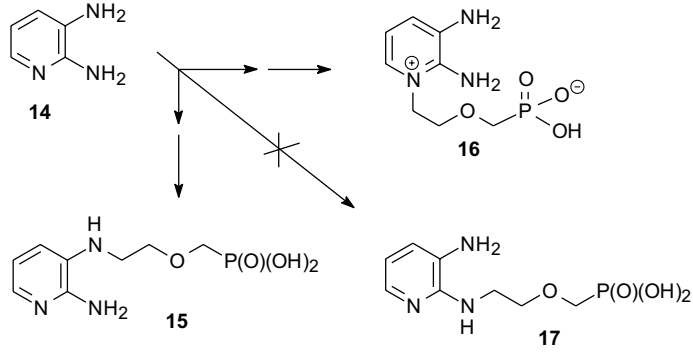
agrees with the data for compound **9** ($\lambda_{max}$ 267 nm) while the $\lambda_{max}$ value of the isomeric 4-amino-2-methylaminopyrimidine as a model for the regioisomer of compound **9** ($\lambda_{max}$ 277 nm at pH 1)[21] significantly differs from our experimental value. Similarly, the UV spectra of the compound **10** ($\lambda_{max}$ 275 nm at pH 1 and 7) are in accord with the *N*1-isomer and differ substantially from those of 2,4-diamino-6-methylaminopyrimidine (($\lambda_{max}$ 280 nm at pH 1 and 270 nm at pH 7)[22]. The low electrophoretic mobility of compounds **9** and **10** is comparable with the value for compound **5**. All these arguments corroborate the quaternary structures bearing the side chain at *N*1-position.

A different situation arose in the application of the above reaction with compound **3** to 2-aminopyrimidine (**11**) and/or its *N*2-dimethylaminomethylene derivative **12** (prepared by the reaction of **11** with dimethylformamide dimethyl acetal): the cleavage of the intermediate with $BrSiMe_3$ and subsequent hydrolysis of the silyl ester groups (in the case of **12** followed by aqueous ammonia treatment) gave 2-{[2-(phosphonomethoxy)ethyl]amino}pyrimidine (**13**) as the only product. Its structural assignment is based on NMR spectra (*vide infra*); also the UV spectrum ($\lambda_{max}$ 232 and 314 nm) corresponds to that structure. Furthermore, the electrophoretic mobility of compound **13** is substantially higher compared to compounds **5**, **9** and **10** as expected for the structure lacking the positive charge which equilibrates the negative charges of the phosphonate residue. The formation of *exo*-*N*2 derivative could be interpreted by direct alkylation of the imino form in the 2-amino derivative **11**. In the case of amidine **12**, the mechanism implies quaternization of the amidine α-nitrogen atom followed by hydrolysis of the amidinium salt. Alternatively, the formation of the non-quaternized *exo*-*N*2 derivative **13** both from **11** and **12** could be explained by quaternization at N1 followed by Dimroth type rearrangement (ring opening–ring closure reaction at alkaline pH).

Generally, compounds with zwitterionic character (**5**, **9**, **10**, **16**) elute from the strongly basic anion exchanger already with very diluted (≈0.02 M) acetic acid solutions while the derivatives with the common phosphonomethyl character (**13**, **15**) are eluted only by the solutions of a higher ionic strength of acetic acid[23].

The reaction of the compound **3** with 2,3-diaminopyridine (**14**) afforded two reaction products which were separated by silica gel chromatography and obtained as light-sensitive oils. Their treatment with $BrSiMe_3$ and hydrolysis gave isomeric 2-(phosphonomethoxy)ethyl derivatives which were purified by anion exchange chromatography: the *N*3-isomer, 2-amino-3-{[2-phosphonomethoxy)ethyl]amino}pyridine **15** and 1-[2-(phosphono-

methoxy)ethyl] derivative **16** (Scheme 3). Their structures follow from the NMR spectra (*vide infra*). The arguments are sustained by their electrophoretic behaviour: the quaternary character of compound **16** is supported by its low electrophoretic mobility which is comparable with compounds **5** and **10**, while the mobility of compound **15** which corresponds to the structurally related compound **13** clearly reflects the double dissociation of the phosphonate group at weakly alkaline pH and excludes the quaternary structure bearing an expressed positive charge which partly neutralizes the phosphonate residue. The reaction of the base **14** with methyl iodide was reported to proceed also with the formation of two products: *N*1-substituted (quaternary) methyl derivative and 2-amino-3-(methylamino)pyridine[24]. The presence of a regioisomer **17** was not detected.




SCHEME 3

*NMR Discussion*

To obtain additional arguments for the assignment of signals and structural interpretation of NMR spectra of the isomeric products, we have also investigated the NMR spectra of the corresponding heterocyclic bases (Table I). The presence of the phosphonomethoxy group in the 1-[2-(phosphonomethoxy)ethyl] (PME) derivatives is manifested by additional spin–spin couplings $^{2}J$(P,H) in $^{1}$H NMR or $^{1}J$(P,C) and $^{3}J$(P,C) in $^{13}$C NMR spectra. The character of signals (doublets of $CH_2$–P and C-2′ carbons in proton-decoupled $^{13}$C NMR spectra) and different splitting (150–165 and 10–13 Hz, respectively) allow unequivocal assignment of carbon signals $CH_2$–P and C-2′ and their distinguishing from C-1′.

Main structural problem in the series of compounds **5**, **9**, **10**, **13**, **15** and **16** consists in the determination of the PME-group location at the

TABLE I
$^1$H and $^{13}$C NMR data of starting pyrimidine and pyridine bases

| Compound | Solvent[a] | $^1$H (coupling constants) | | | | | $^{13}$C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H-2 | H-4 | H-5 | H-6 | $NH_2$ | C-2 | C-3 | C-4 | C-5 | C-6 |
| **2** | S | – | – | 5.67 d (5.7) | 7.61 d (5.7) | 5.78 (2 H) 6.21 (2 H) | 163.54 | – | 164.19 | 95.17 | 156.07 |
| | W | – | – | 6.02 d (6.0) | 7.76 d (6.0) | – | 165.17 | – | 167.41 | 99.75 | 158.68 |
| **7** | S | 7.80 d (1.0) | – | 5.36 (1.0) | – | 6.00 (4 H) | 158.00 | – | 163.68 | 82.76 | 163.68 |
| | W | 7.96 bs | – | 5.71 bs | – | – | 166.26 | – | 160.59 | 87.46 | 160.59 |
| **8** | S | – | – | 4.84 s | – | 5.28 (2 H) 5.50 (4 H) | 163.26 | – | 164.70 | 75.03 | 164.70 |
| | W | – | – | 5.24 s | – | – | 167.69 | – | 167.73 | 79.31 | 167.73 |
| **11** | S | – | 8.20 d (4.8) | 6.53 t (4.8) | 8.20 d (4.8) | 6.56 (2 H) | 163.76 | – | 158.16 | 110.26 | 158.16 |
| | W | – | 8.31 d (5.0) | 6.79 t (5.0) | 8.31 d (5.0) | – | 164.83 | – | 161.36 | 114.39 | 161.36 |
| **14** | S | – | 7.26 dd (4.9; 1.6) | 6.35 dd (7.4; 4.9) | 6.67 dd (7.4; 1.6) | 4.61 (2 H) 5.30 (2 H) | 148.65 | 130.03 | 118.34 | 113.33 | 135.18 |
| | W | – | 7.56 dd (5.2; 1.5) | 6.75 dd (4.6; 5.2) | 7.13 dd (4.6; 1.5) | – | 145.63 | 131.99 | 127.00 | 118.78 | 140.51 |

[a] S, ($CD_3SOCD_3$); W, $D_2O$.

heteroaromatic ring. Their poor solubility makes impossible to apply the method based on vicinal couplings $J$(C,H) of α-methylene protons of substituent observable in proton-coupled $^{13}$C NMR spectra (*e.g.* ref.[25]). The structural arguments are therefore based on chemical shifts, different symmetry of alternative structures, NOE and/or spin–spin coupling in $^{1}$H NMR spectra.

The *N*1-position of the PME-group in compound **5** was derived from difference 1D NOE spectra. Saturation of C(1′)$H_2$ signal at δ 4.13 resulted in the 8% NOE enhancement of doublet at δ 7.67 belonging to proton H-6. Similar approach has been applied in case of compound **9**, where saturation of C(1′)$H_2$ signal at δ 4.28 resulted in the 12% NOE enhancement of singlet at δ 8.23 belonging to proton H-2 in accordance with proposed structure **9**.

The arguments following from the symmetry of the molecule have been used for compounds **10** and **13**. Two-fold symmetry axis (manifested by the chemical shift equivalence of protons H-4, H-6 as well as carbons C-4, C-6) and spin–spin coupling between H-1′ and NH (observed in $^{1}$H NMR spectrum of compound **13** taken in DMSO) indicate the substitution by the PME-chain at the amino group in position 2. On the other hand, four non-equivalent carbon signals of the substituted pyrimidine ring in compound **10** exclude the substitution at the exocyclic *N*2-amino group. The absence of the NOE at singlet of H-5 (at δ 5.15) under irradiation of N-$CH_2$ signal at δ 4.18 and close similarity of the $^{13}$C chemical shift of C-1′ (δ 49.29) with above discussed compounds **5** and **9** strongly support the structure **10** with the 2-(phosphonomethoxy)ethyl group in position 1.

Reaction of 2,3-diaminopyridine (**14**) provided two isomeric products: lowfield position of carbon C-1′ (δ 57.04) and corresponding methylene protons (δ 4.46) in compound **16** and, particularly, 9% NOE enhancement of the H-6 signal (at δ 7.46) observed at irradiation of H-1′ protons (at δ 4.46) indicate that the PME-group is located at the position N1. The isomeric compound **15** showed an upfield shift of C-1′ (δ 45.96) and H-1′ (δ 3.21) and 8% NOE at H-4 (δ 6.98) under irradiation of H-1′ protons in accordance with the substitution at C(3)–$NH_2$ group.

*Biological Activity*

The free phosphonates **5**, **9**, **10**, **13**, **15** and **16** were screened for their antiviral activity against DNA viruses and retroviruses under standard *in vitro* conditions (*cf.* ref.[10]). Also their cytostatic activity under *in vitro* conditions was examined in L-1210, P388 and CEM cell lines. No activity was encountered in these systems with any of the compounds tested[26,27].

## EXPERIMENTAL

Unless otherwise stated, the solvents were evaporated at 40 °C/2 kPa and the compounds were dried overnight at 2 kPa over $P_2O_5$. Melting points were determined on a Kofler block and are uncorrected. TLC was performed on Silufol UV254 plates (Kavalier Votice, Czech Republic) in system S1, chloroform–ethanol (4 : 1). Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogencarbonate (TEAB) at pH 7.5; the electrophoretical mobilities were referenced to uridine 3′-phosphate.

*NMR spectra* were recorded on an FT NMR spectrometer Varian UNITY-500. $^1$H NMR spectra (at 500 MHz) were taken in $D_2O$ (with 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt as internal reference) and $CD_3SOCD_3$ solutions (referenced to the signal of solvent at δ 2.50). Chemical shifts and coupling constants were obtained by the first order analysis of spectra. Proton 1D NOE spectra were obtained by selective irradiation during 5 s before data acquisition and subtraction of two spectra taken with irradiation frequency placed on- and off-resonance. $^{13}$C NMR spectra (at 125.7 MHz) were measured in $D_2O$ (referenced to TSP) and/or $CD_3SOCD_3$ (referenced to the solvent signal at δ 39.7) using either "attached proton test" or standard broadband proton decoupling pulse sequence.

*Mass spectra* were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix).

*UV absorption spectra* were measured on a Beckman DU-65 spectrometer in aqueous solutions.

### Materials

Bromotrimethylsilane, 2-aminopyrimidine, 2,4-diaminopyrimidine, 2,4,6-triaminopyrimidine, 2,4-diamino-6-hydroxypyrimidine and 2,3-diaminopyridine were purchased from Sigma–Aldrich (Prague, Czech Republic), 4,6-diaminopyrimidine from Lachema (Brno, Czech Republic). Compound **3** was synthesized according to the literature[10]. Dimethylformamide dineopentyl acetal was prepared according to ref.[15]. Dimethylformamide was distilled from phosphorus pentoxide *in vacuo* and stored over molecular sieves.

### Purification of the Phosphonates by Dowex 1 X 2 Column Chromatography. General Procedure

Unless stated otherwise, 100 ml columns of Dowex 1 X 2 (100–200 mesh, acetate form, prewashed with water) were used. The sample was dissolved in water (20–25 ml), alkalized with concentrated aqueous ammonia to pH 9–9.5 and applied on the column. Elution with water (3 ml/min) was continued till the UV absorption (254 nm) of the eluate dropped to the initial value. The column was then eluted either with water to afford the (zwitterionic) product with retention, with 0.02 M acetic acid (3 ml/min, fractions 30 ml), or with a linear gradient of acetic acid (0–0.5 mol/l, 1 l each) as indicated.

### 2-[(Dimethylaminomethylene)amino]pyrimidine (**12**)

2-Aminopyrimidine (**11**; 10.5 g, 0.1 mol) in dimethylformamide dimethyl acetal (80 ml) was stirred at 100 °C till dissolution (15 min) and left to stand overnight at room temperature. The mixture was taken down to dryness *in vacuo*, codistilled with toluene (2 × 25 ml) and

the residue was crystallized from toluene (ether added to turbidity). Yield 13.6 g (90.5%) of compound **12**, m.p. 105 °C. For $C_7H_{10}N_4$ (150.2) calculated: 55.98% C, 6.71% H, 37.31% N; found: 56.13% C, 6.75% H, 37.10% N. $^1$H NMR ($CD_3SOCD_3$): 3.02 d, 3 H, $J$($CH_3$,CH) = 0.6 ($CH_3$); 3.11 s, 3 H ($CH_3$); 6.92 t, 1 H, $J$(5,4) = $J$(5,6) = 4.7 (H-5); 8.47 d, 2 H, $J$(4,5) = 4.7 (H-4 and H-6); 8.60 bs, 1 H (=CH-). $^{13}$C NMR ($CD_3SOCD_3$): 34.55 and 40.45 (2 × $CH_3$); 114.80 (C-5); 158.21 (C-4 and C-6); 158.25 (=CH-); 166.69 (C-2).

4-Amino-2-[(dimethylaminomethylene)amino]pyrimidine (**6**)

2,4-Diaminopyrimidine (**2**; 2.75 g, 25 mmol) and dimethylformamide dineopentyl acetal (15 ml) were stirred in DMF (30 ml) for 16 h at 80 °C and taken down to dryness at 40 °C/14 Pa. The residue was treated with ethanol (25 ml) and a few pieces of dry ice for 20 min and evaporated. The residue was stirred with ethanol (25 ml), ether (50 ml) added, and the product was filtered, washed with ether and dried *in vacuo*. Yield 4.6 g (83.5%), m.p. 197–198 °C. For $C_{10}H_{16}N_6$ (220.3) calculated: 54.53% C, 7.32% H, 38.15% N; found: 54.88% C, 7.55% H, 38.40% N. Mass spectrum: $MH^+$ 221.1. $^1$H NMR ($CD_3SOCD_3$): 2.97 bs, 3 H ($CH_3$); 3.07 s, 3 H ($CH_3$); 6.02 d, 1 H, $J$(5,6) = 5.5 (H-5); 6.70 bs, 2 H ($NH_2$); 7.88 d, 1 H, $J$(6,5) = 5.5 (H-6); 8.48 bs, 1 H (N=CH-N). $^{13}$C NMR ($CD_3SOCD_3$): 34.47 and 40.48 (2 × $CH_3$); 104.06 (C-5); 156.18 (C-6); 157.85 (N=CH-N); 163.65 (C-4); 167.99 (C-2).

Hydrogen {[2-(2,4-Diaminopyrimidin-1-io)ethoxy]methyl}phosphonate (**5**)

*Method A*. The mixture of 2,4-diaminopyrimidine (**2**; 5.0 g, 45.4 mmol) and compound **3** (20 ml) was stirred in Ar atmosphere for 16 h at 80 °C. Ether (150 ml) was slowly added under stirring and the solvent decanted. The residue was stirred in ether (100 ml), filtered, washed with ether and dried *in vacuo* for 20 min at ambient temperature. The hygroscopic residue (homogeneous by paper electrophoresis, $E_{Up}$ –0.48) was dissolved in acetonitrile (100 ml), $BrSiMe_3$ (10 ml) was added and the mixture was left to stand overnight at room temperature. The solvents were stripped down *in vacuo*, the residue codistilled with acetonitrile (2 × 25 ml) and water (50 ml) was added to the residue. After 10 min, the solution was slightly alkalized with concentrated aqueous ammonia and evaporated *in vacuo*. The residue was applied in concentrated aqueous solution onto a column (150 ml) of Dowex 50 X 8 ($H^+$-form) and the column eluted with water (3 ml/min); the elution was followed by continuous measurement of UV absorption of the eluate at 254 nm. After removal of the neutral UV-absorbing fraction the column was eluted with 2.5% aqueous ammonia and the UV-absorbing fraction collected. It was taken down *in vacuo*, redissolved in water (20 ml), brought to pH 9–10 by concentrated aqueous ammonia and applied on a column (100 ml) Dowex 1 X 2 (acetate form) thorougly prewashed with water. The column was processed as described in General Procedure and the product was eluted with 0.02 M acetic acid. The UV-absorbing eluate was taken down *in vacuo*, the residue was codistilled with water (2 × 25 ml) and the residue crystallized from water. Yield 5.10 g (42.4%) of compound **5**, m.p. >280 °C (water). $E_{Up}$ 0.63. For $C_7H_{13}N_4O_4P{\cdot}H_2O$ (266.2) calculated: 31.59% C, 5.68% H, 21.05% N, 11.64% P; found: 31.73% C, 5.76% H, 21.27% N, 11.88% P. UV [$\lambda_{max}$ ($\varepsilon_{max}$)] (pH 2, pH 7): 270 (6 200). Mass spectrum: $MH^+$ 249.1. $^1$H NMR ($CD_3SOCD_3$): 3.37 d, 2 H, $J$($CH_2$,P) = 6.4 ($CH_2$P); 3.77 t, 2 H, $J$(2′,1′) = 6.5 (H-2′); 4.20 t, 2 H, $J$(1′,2′) = 6.5 (H-1′); 6.02 d, 1 H, $J$(5,6) = 7.2 (H-5); 7.83 d, 1 H, $J$(6,5) = 7.2 (H-6); 7.90 bs, 1 H (NH); 7.98 bs, 1 H (NH). $^1$H NMR ($D_2O$): 3.65 d, 2 H, $J$($CH_2$,P) = 8.7 ($CH_2$P); 3.93 t, 2 H, $J$(2′,1′) = 5.0 (H-2′); 4.13 t, 2 H, $J$(1′,2′) =

5.0 (H-1′); 6.23 d, 1 H, *J*(5,6) = 7.3 (H-5); 7.67 d, 1 H, *J*(6,5) = 7.3 (H-6). $^{13}$C NMR ($D_2O$): 54.93 (C-1′); 70.57 d, *J*($CH_2$,P) = 156.6 ($CH_2$P); 72.42 d, *J*(2′,P) = 11.9 (C-2′); 102.08 (C-5); 149.48 (C-6); 158.55 (C-4); 167.12 (C-2).

*Method B.* Compound **6** (3.3 g, 15 mmol) was stirred with compound **3** (20 ml) for 16 h at 100 °C and precipitated with ether (100 ml). The product was filtered, washed with ether and dried *in vacuo*. Acetonitrile (70 ml) and $BrSiMe_3$ (10 ml) were added and the mixture was left to stand overnight at room temperature. The volatiles were taken down *in vacuo*, the residue was dissolved in water (50 ml) and the solution was left to stand for 1 h at ambient temperature. The mixture was alkalized with concentrated aqueous ammonia and evaporated. The chromatography on Dowex 50 was performed as described in method A (the ammonia eluate was left to stand overnight prior to evaporation). Further work-up and chromatography on Dowex 1 column was performed as described in method *A*. Yield 1.9 g (47.3%) of compound **5**, identical with the material prepared by method *A*.

Hydrogen {2-[(2,6-Diaminopyrimidin-1-io]ethoxy]methyl}phosphonate (**9**)

Compound **9** was prepared from 2.5 g (19.8 mmol) 2,6-diaminopyrimidine (**7**) as described for compound **5** (method *A*). Yield 1.70 g (32.3%), m.p. 187 °C (80% aqueous ethanol). $E_{Up}$ 0.60. For $C_7H_{13}N_4O_4P{\cdot}H_2O$ (266.2) calculated: 31.59% C, 5.68% H, 21.05% N, 11.64% P; found: 31.80% C, 5.67% H, 21.23% N, 11.54% P. UV [$\lambda_{max}$ ($\varepsilon_{max}$)] (pH 2, pH 7): 267 (10 200). $^1$H NMR ($CD_3SOCD_3$): 3.38 d, 2 H, *J*($CH_2$,P) = 6.3 ($CH_2$P); 3.78 t, 2 H, *J*(2′,1′) = 6.7 (H-2′); 4.31 t, 2 H, *J*(1′,2′) = 6.7 (H-1′); 5.60 s, 1 H (H-5); 7.46 bs, 1 H and 7.64 bs, 1 H ($NH_2$); 8.32 s, 1 H (H-2); 9.00 vbs, 2 H ($NH_2$). $^1$H NMR ($D_2O$): 3.66 d, 2 H, *J*($CH_2$,P) = 8.6 ($CH_2$P); 3.93 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 4.28 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 5.86 s, 1 H (H-5); 8.23 s, 1 H (H-2). $^{13}$C NMR ($D_2O$): 52.60 (C-1′); 70.61 d, *J*($CH_2$,P) = 156.7 ($CH_2$P); 72.28 d, *J*(2′,P) = 11.7 (C-2′); 85.86 (C-5); 155.70 (C-2); 158.02 (C-6); 165.31 (C-4).

Hydrogen {2-[(2,4,6-Triaminopyrimidin-1-io)ethoxy]methyl}phosphonate (**10**)

The mixture of 2,4,6-triaminopyrimidine (**8**; 3.0 g, 24 mmol), compound **3** (7 ml) and DMF (7 ml) was stirred for 14 h at 100 °C, taken down *in vacuo* and codistilled with DMF (2 × 20 ml). The residue was treated with acetonitrile (60 ml) and $BrSiMe_3$ (7 ml) overnight and worked up as described for compound **5** (method *A*). Elution with 0.02 M acetic acid afforded 1.85 g (29%) of compound **10**, m.p. 260 °C (decomp.) (80% aqueous ethanol–ether). $E_{Up}$ 0.48. For $C_7H_{13}N_5O_4P$ (263.2) calculated: 31.95% C, 5.36% H, 26.61% N, 11.77% P; found: 32.20% C, 5.62% H, 26.81% N, 11.85% P. UV [$\lambda_{max}$ ($\varepsilon_{max}$)] (pH 2, pH 7): 275 (15 500). Mass spectrum: $MH^+$ 264.0. $^1$H NMR ($CD_3SOCD_3$): 3.39 d, 2 H, *J*($CH_2$,P) = 6.4 ($CH_2$P); 3.76 t, 2 H, *J*(1′,2′) = 6.2 (H-1′); 4.18 t, 2 H, *J*(2′,1′) = 6.2 (H-2′); 5.15 s, 1 H (H-5); 6.90 vbs, 2 H ($NH_2$); 7.90 bs, 4 H (2 × $NH_2$). $^1$H NMR ($D_2O$): 3.62 d, 2 H, *J*($CH_2$,P) = 8.5 ($CH_2$P); 3.93 t, 2 H, *J*(2′,1′) = 4.8 (H-2′); 4.16 t, 2 H, *J*(1′,2′) = 4.8 (H-1′); 5.41 s, 1 H (H-5).$^{13}$C NMR ($D_2O$): 49.29 (C-1′); 70.95 d, *J*($CH_2$,P) = 155.3 ($CH_2$P); 73.01 d, *J*(2′,P) = 11.7 (C-2′); 79.53 (C-5); 158.66 and 159.34 (C-2 and C-6); 165.84 (C-4).

2-{[2-(Phosphonomethoxy)ethyl]amino}pyrimidine (**13**)

*a*) Compound **12** (3.0 g, 20 mmol) and compound **3** (15 ml) were stirred for 2 h at 100 °C and the mixture was worked up as described for compound **5** (method *A*). After deionization, the product was eluted from Dowex 1 column by 0.5 M acetic acid. Yield 2.15 g

(42.8%) of compound **13**, m.p. 199 °C (ethanol–ether). $E_{Up}$ 1.00. For $C_7H_{12}N_3O_4P \cdot H_2O$ (251.2) calculated: 33.47% C, 5.62% H, 16.73% N, 12.33% P; found: 33.60% C, 5.56% H, 17.01% N, 12.22% P. UV [$\lambda_{max}$ ($\varepsilon_{max}$)] (pH 2, pH 7): 232, 310 (3 200). Mass spectrum: $MH^+$ 234.0. $^1$H NMR ($CD_3SOCD_3$): 3.43 q, 2 H, *J*(1′,2′) = *J*(1′,NH) = 6.1 (H-1′); 3.56 d, 2 H, *J*($CH_2$,P) = 8.7 ($CH_2$P); 3.61 t, 2 H, *J*(2′,1′) = 6.1 (H-2′); 6.57 t, 1 H, *J*(5,4) = *J*(5,6) = 4.7 (H-5); 7.02 bt, 1 H, *J*(1′,NH) = 6.1 (NH); 8.26 d, 2 H, *J*(4,5) = *J*(6,5) = 4.7 (H-4, H-6). $^1$H NMR ($D_2O$): 3.68 d, 2 H, *J*($CH_2$,P) = 8.7 ($CH_2$P); 3.71 m, 2 H (H-1′); 3.82 m, 2 H (H-2′); 6.99 t, 1 H, *J*(5,4) = *J*(5,6) = 5.4 (H-5); 8.52 d, 2 H, *J*(4,5) = 5.4 (H-4, H-6). $^{13}$C NMR ($D_2O$): 43.64 (C-1′); 69.88 d, *J*($CH_2$,P) = 156.8 ($CH_2$P); 73.92 d, *J*(2′,P) = 11.2 (C-2′); 113.58 (C-5); 161.12 (C-4 and C-6); 162.95 (C-2).

*b*) 2-Aminopyrimidine (**11**; 3.0 g, 31.6 mmol) was treated for 24 h at 100 °C with compound **3** (15 ml). The standard work-up gave 1.6 g (20.3%) of compound **13** which was eluted with 0.35–0.40 M acetic acid (linear gradient, 1 l water, 1 l 0.5 M acetic acid). The product was identical by NMR and UV spectra with the material prepared by previous procedure.

2-Amino-3-{[2-(phosphonomethoxy)ethyl]amino}pyridine (**15**) and
Hydrogen {2-[(2,3-Diaminopyridin-1-io)ethoxy]methyl}phosphonate (**16**)

The mixture of 2,3-diaminopyridine (**14**; 5.0 g, 45.9 mmol) and compound **3** (25 ml) was stirred for 24 h at 80 °C and precipitated with ether as described for compound **5**. The precipitate was chromatographed on silica gel column (300 ml) in chloroform to give two fractions ($R_F$ 0.37 and 0.16, respectively, S1) as red light-sensitive oils. The products were dried *in vacuo* over $P_2O_5$ and treated each with $BrSiMe_3$ (5 ml) in acetonitrile (50 ml) overnight. The reaction mixtures were worked up as described for compound **5** (method *A*). Purification of the desalted mixture from the diester $R_F$ 0.37 on Dowex 1 column (elution with 0.02 M acetic acid) gave 1.8 g (15.9%) of the *N*3-isomer **15**, $E_{Up}$ 0.82 (blue fluorescence), m.p. 259 °C (water). For $C_8H_{14}N_3O_4P$ (247.2) calculated: 38.87% C, 5.71% H, 17.00% N, 12.53% P; found: 38.80% C, 5.77% H, 16.83% N, 12.58% P. Mass spectrum: $MH^+$ 248.1. $^1$H NMR ($CD_3SOCD_3$): 3.12 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.59 d, 2 H, *J*($CH_2$,P) = 7.0 ($CH_2$P); 3.85 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 6.63 dd, 1 H, *J*(5,4) ≈ 7.5, *J*(5,6) ≈ 6.0 (H-5); 6.68 dd, 1 H, *J*(4,5) ≈ 7.5, *J*(4,6) ≈ 1.0 (H-4); 7.14 dd, 1 H, *J*(6,5) ≈ 6.0, *J*(6,4) ≈ 1.0 (H-6); 8.42 bs, 2 H ($NH_2$). $^1$H NMR ($D_2O$): 3.67 d, 2 H, *J*($CH_2$,P) = 10.6 ($CH_2$P); 3.21 m, 2 H (H-1′); 3.86 m, 2 H (H-2′); 6.82 dd, 1 H, *J*(5,4) = 8.0, *J*(5,6) = 6.3 (H-5); 6.98 dd, 1 H, *J*(4,5) = 8.0, *J*(4,6) = 1.3 (H-4); 7.27 dd, 1 H, *J*(6,5) = 6.3, *J*(6,4) = 1.3 (H-6). $^{13}$C NMR ($D_2O$): 45.96 (C-1′); 70.45 d, *J*($CH_2$,P) = 151.0 ($CH_2$P); 73.20 d, *J*(2′,P) = 12.7 (C-2′); 116.73, 121.51 and 126.00 (C-4, C-5 and C-6); 135.90 and 148.89 (C-2 and C-3).

The second fraction from the silica gel chromatography ($R_F$ 0.16) gave after the purification by Dowex 1 chromatography (elution with 0.02 M acetic acid) product which was repeatedly coevaporated with water and treated with acetone and ether to afford amorphous quaternary compound **16** (1.9 g, 16.8%), slightly contaminated with the *N*3-isomer **15**. Rechromatography on Dowex 1 X 2 under the same conditions removed this impurity ($E_{Up}$ 0.43). For $C_8H_{14}N_3O_4P$ (247.2) calculated: 38.87% C, 5.71% H, 17.00% N, 12.53% P; found: 39.17% C, 5.48% H, 16.78% N, 12.86% P. Mass spectrum: $MH^+$ 248.1. $^1$H NMR ($CD_3SOCD_3$): 3.44 d, 2 H, *J*($CH_2$,P) = 7.0 ($CH_2$P); 3.90 t, 2 H, *J*(2′,1′) = 6.0 (H-2′); 4.48 t, 2 H, *J*(1′,2′) = 6.0 (H-1′); 6.04 bs, 2 H ($NH_2$); 6.64 dd, 1 H, *J*(5,4) = 7.7, *J*(5,6) = 6.6 (H-5); 6.88 dd, 1 H, *J*(4,5) = 7.7, *J*(4,6) = 1.3 (H-4); 7.32 dd, 1 H, *J*(6,5) = 6.6, *J*(6,4) = 1.3 (H-6); 8.96 bs ($NH_2$). $^1$H NMR

($D_2O$): 3.63 d, 2 H, $J(CH_2,P)$ = 8.7 ($CH_2P$); 4.04 m, 2 H (H-1′); 4.46 m, 2 H (H-2′); 6.83 dd, 1 H, $J(5,4)$ = 7.8, $J(5,6)$ = 6.7 (H-5); 7.28 dd, 1 H, $J(4,5)$ = 7.8, $J(4,6)$ = 1.4 (H-4); 7.46 dd, 1 H, $J(6,5)$ = 6.7, $J(6,4)$ = 1.4 (H-6). $^{13}C$ NMR ($D_2O$): 57.04 (C-1′); 70.59 d, $J(CH_2,P)$ = 156.3 ($CH_2P$); 72.54 d, $J(2',P)$ = 11.7 (C-2′); 116.96, 126.74 and 133.44 (C-4, C-5 and C-6); 135.87 and 150.11 (C-2 and C-3).

X-Ray Analysis of Compound **5**

Compound **5**·$H_2O$, $C_8H_{15}N_4O_4P$, $M$ = 266.20, triclinic, space group $P\bar{1}$ (No. 2), $a$ = 7.476(1), $b$ = 7.562(1), $c$ = 11.217(1) Å, $\alpha$ = 90.86(1), $\beta$ = 92.36(1), $\gamma$ = 117.19(1)°, $V$ = 563.2(1) Å$^3$, $F(000)$ = 280, $D_c$ = 1.570 g/cm$^3$ for $Z$ = 2. A colourless plate of the dimensions 0.6 × 0.4 × 0.15 mm (from hot water) was measured on a CAD4 diffractometer at 150.0(1) K (MoK$\alpha$ radiation, $\lambda$ = 0.71073 Å). From a total of 2 480 independent reflections measured in the range $h$ = –9 to 8, $k$ = 0 to 9, $l$ = –14 to 14, 2 249 were regarded as observed according to the $I > 2\sigma(I)$ criterion. Three standard reflections monitored every 1 h showed 1.9% total decay. Absorption was neglected ($\mu$ = 0.263 mm$^{-1}$). The structure was solved by direct methods (SIR97, ref.[30]) and refined by full-matrix least squares based on $F^2$ (SHELXL93, ref.[31]) with hydrogen atoms isotropic, all other atoms anisotropic. The refinement converged to $R$ = 0.0290, $wR$ = 0.0792, $GOF$ = 1.073 for 214 parameters. The final difference map displayed no peaks of chemical significance. Standard SHELX output files are available from the author (J. P.) on request. Crystallographic data for the structure **5** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-112393. Copies of the data can be obtained free of charge on application to CCDC, e-mail: deposit@ccdc.ca.ac.uk.

*This work was supported by grants of the Grant Agency of the Czech Republic (No. 203/96/K001, No. 203/96/0497 and No. 203/97/0242), by Gilead Sciences (Foster City, CA, U.S.A.) and by the General Health Insurance Agency of the Czech Republic. The cytostatic activity was examined by Dr I. Votruba of This Institute, the antiviral effect by Drs R. Snoeck, G. Andrei, J. Balzarini and E. De Clercq at the Rega Institute, Katholic University, Leuven (Belgium). The UV spectra were measured by Dr E. Tloušťová, mass spectra by Dr J. Kohoutová, both from This Institute. The excellent technical assistance of Ms B. Nováková is gratefully acknowledged.*

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