SYNTHESIS AND BIOLOGICAL EFFECTS OF N-(2-PHOSPHONOMETHOXYETHYL) DERIVATIVES OF DEAZAPURINE BASES

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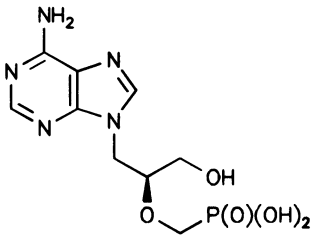
Analogs of antiviral 9-(2-phosphonomethoxyethyl)adenine (PMEA, *II*), containing modified purine bases 1-deazaadenine (*VII*), 3-deazapurine (*XI*), 7-deaza-7-cyanoadenine (*XIIIb*) and 3-deazaguanine (*XXIb*), were prepared by alkylation of the heterocyclic bases with bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*) in dimethylformamide in the presence of sodium hydride or cesium carbonate. The obtained protected derivatives were deblocked with bromotrimethylsilane to give the phosphonic acids. 3-DeazaPMEG (*XXIb*) is active against DNA viruses and exhibits a marked cytostatic effect against L-1210 leukemia.

Systematic investigations of nucleoside analogs have shown that usually these compounds act in vivo only after phosphorylation leading to the corresponding 5′-nucleotides which are the antimetabolites proper. However, a direct application of these nucleotides to organism usually fails due to dephosphorylation in the blood plasma and/or during permeation through the cell membrane. The in vivo instability of the phosphomonoester bond has directed our attention to isopolar nucleotide analogs which would not undergo the in vivo enzymatic dephosphorylation[1].

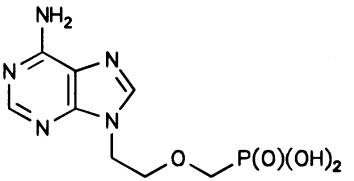
From the viewpoint of isosteric and isopolar similarity, O-phosphonomethyl derivatives of nucleosides appear to be promising. These compounds contain methylene group bonded between the phosphorus atom and the hydroxyl oxygen atom of the nucleoside sugar moiety. Extraordinarily interesting, however, were not the mentioned nucleoside derivatives but the analogous phosphonomethyl compounds derived from acyclic nucleosides (acyclic analogs of nucleotides), particularly N-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl) and N-(2-phosphonomethoxyethyl) derivatives of heterocyclic bases (HPMP derivatives, *I*, and PME derivatives, *II*, respectively) which exhibit high antiviral activity[2-5].

So far, structure–activity studies have shown that the biological activity in the PME-series is limited only to purine derivatives substituted with a side chain in position N-9. The compounds have a high in vitro as well as in vivo activity against herpes viruses, iridoviruses, adenoviruses and poxviruses[6,7]. Derivatives of adenine, guanine and 2,6-diaminopurine are also active against retroviruses (HIV-1, HIV-2, SIV, FIV)[6,8-11] and

suppress multiplication of human hepatitis virus B (ref.[12]). PMEA is a more potent antiretroviral than AZT against Moloney sarcoma virus in mice[9]. PME derivatives also exhibit a significant effect on L-1210 mice leukemia cells[13].



HPMPA (*I*)

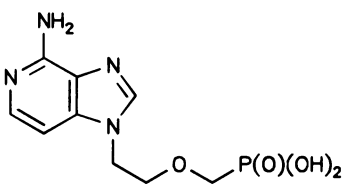


PMEA (*II*)

It appeared that this biological effect is very strongly limited by the character of the base: not only the PME derivative of hypoxanthine and xanthine but also the 2-hydroxyadenine, 2-methylthio- or 2-methyladenine and other derivatives of 6- or 8-substituted purine bases have no marked activity against the mentioned DNA viruses and retroviruses. Inactive are also compounds derived from pyrimidine bases (cytosine, uracil and thymine)[6].

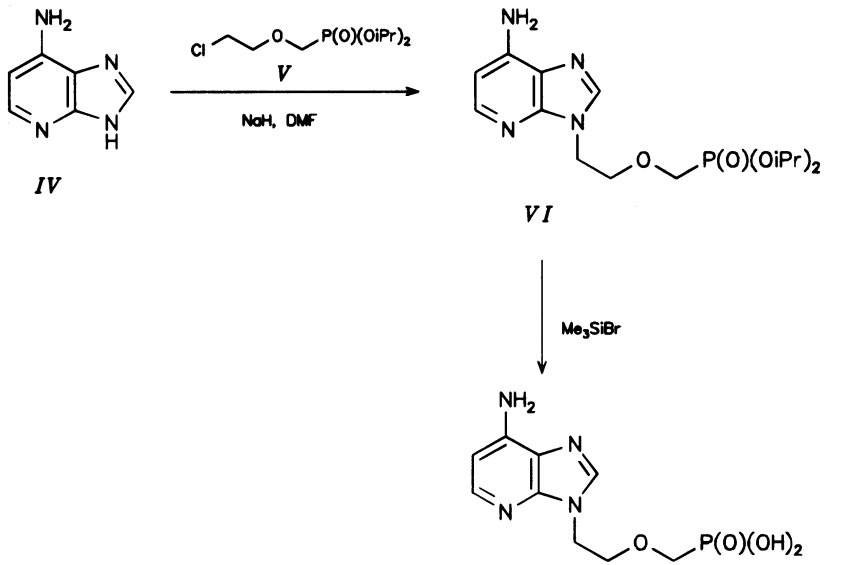
It is well known that replacement of the –N= atom in imidazole or pyrimidine ring of purines by a methine group brings about no change in the steric demands of the formed system. 1-, 3- or 7-Deazapurine nucleosides (or bases) often have marked antimetabolic character: 3-deazaadenosine and its arabino-analog exhibit antiviral activity[14,15], 1-deazaadenosine affects blood coagulation[16], 7-deazaadenosine and its 7-cyano derivative (toyocamycin) are natural antibiotics[17,18], 3-deazaguanine and its nucleosides are potent cytostatics[19].

Recently, we described antiviral effect of N-(3-hydroxy-2-phosphonomethoxypropyl) derivatives derived from deaza analogs of purine bases[20]. Our present paper deals with the synthesis of N-(2-phosphonomethoxyethyl) derivatives of 1-deazaadenine, 3-deazapurine, 7-deaza-7-cyanoadenine and 3-deazaguanine. The preparation of 3-deaza analog *III* was published already earlier[5].



3-deazaPMEA (*III*)

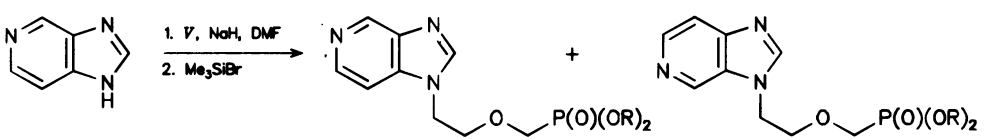
The PME compound derived from 1-deazaadenine was prepared by alkylation of sodium salt of base *IV* with bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*). Using the bis(2-propyl) ester (instead of diethyl ester, employed in the preparation of 3-deazaadenine derivative[5]) improved markedly the yield because there was no concurrent alkylation of the base with the ethyl group generated from the ester $RCH_2P(O)(OEt)_2$ (ref.[21]). As in the case of 3-deazaadenine, the reaction gave predominantly the N^9-isomer *VI* which on treatment with bromotrimethylsilane afforded phosphonic acid *VII* (Scheme 1). The structure of the N^9-isomer was confirmed by comparison of its UV spectra with the literature data[22].



IV *VI* *VII*

SCHEME 1

On the other hand, alkylation of 3-deazapurine (*VIII*) (Scheme 2) afforded a 7 : 3 mixture of the N^9- and N^7-isomers which could not be separated either as diesters *IX* and *X* or as free phosphonates *XI* and *XII*. The assignment and ratio of these position



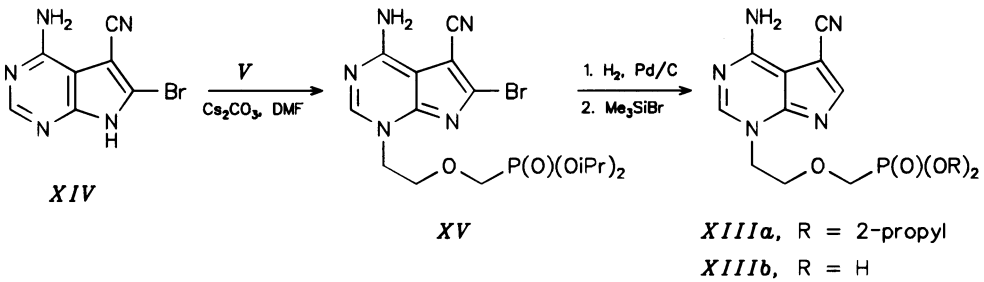
VIII

IX, R = 2-propyl *X*, R = 2-propyl
XI, R = H *XII*, R = H

SCHEME 2

isomers was derived from comparison of their ^{13}C NMR spectra (see Experimental) with those of the hitherto described N-(2,3-dihydroxypropyl) derivatives[23].

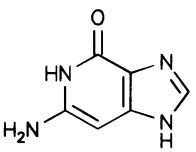
In our recent communication[23] we described the marked catalytic effect of cesium carbonate on alkylation of purine bases (and their deaza derivatives) with oxiranes, alkyl halides and alkyl tosylates. This effect also strongly operated in the preparation of PME derivative of substituted 7-deazaadenine *XIIIb*. In this procedure we decided to make use of the known directive effect of bromine atom in position 6 of the pyrrole ring[24]. Synthon *V* was condensed with 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XIV*) in the presence of cesium carbonate. Nonetheless, we have obtained the protected bromo derivative *XV* as the main reaction product. In the next stage the bromine atom was removed by hydrogenolysis on 10% Pd/C. The diester *XIIIa* was converted to the free acid *XIIIb* by the usual treatment with bromotrimethylsilane (Scheme 3). Similar preferential formation of the N^1-isomer was observed also in the displacement of *p*-toluenesulfonyloxy groups[20,23].



XIV *XV* *XIIIa*, R = 2-propyl; *XIIIb*, R = H

SCHEME 3

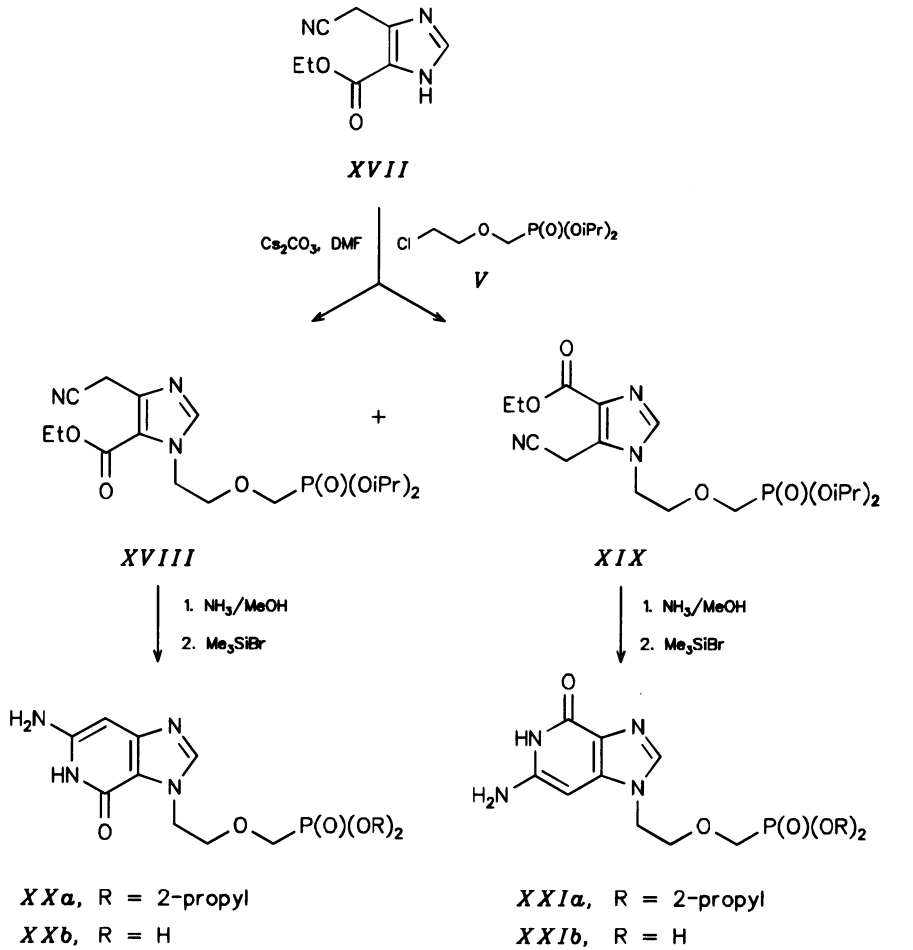
However, the condensation reaction of the base with the synthon, used successfully in all previous cases, failed completely with 3-deazaguanine (*XVI*).



XVI

The reaction mixture did not contain even traces of the desired products. This observation corresponds to our experience with the guanine series. Therefore, we prepared N-(2-phosphonomethoxyethyl)-3-deazaguanine by a procedure analogous to that used by Cook and coworkers[25] in the synthesis of 3-deazaguanosine, i.e., by alkylation of 5(4)-(cyanomethyl)-4(5)-ethoxycarbonylimidazole (*XVII*) followed by formation of the pyrimidine ring on treatment with ammonia.

The imidazole *XVII* was alkylated with synthon *V* under usual conditions (Scheme 4) to give a mixture of N^1- and N^3-substituted products *XVIII* and *XIX* in the respective yields 76% and 16%. The predominant formation of the N^3-isomer *XVIII* was observed for this imidazole system already earlier[25 - 28]. Heating of the obtained intermediates with methanolic ammonia at 110 °C (instead of the described[25] treatment with liquid ammonia) resulted in smooth cyclization to compounds *XXa* and *XXIa*. Deprotection of these diesters with bromotrimethylsilane afforded 3-deaza analogs *XXb* and *XXIb* which were isolated only in a very low overall yield, obviously due to instability of the cyclization products.



SCHEME 4

Structure of the position isomers was unequivocally confirmed on the basis of previously described characteristic UV and ^{13}C NMR spectra (see Experimental)[26].

The 1-deaza derivative *VII*, as well as the 3-deazaadenine derivative *III*, has no effect on the DNA and RNA viruses studied[29]. Also in the group of retroviruses no protective effect of these analogs was observed against cell transformation by HIV-1 and HIV-2. However, certain in vitro effect of 3-deazaadenine derivative *III* on HBV was observed[30]. The 3-deazapurine and toyocamycin analogs *XI* and *XIIIb* showed no effect on the tested viruses. Contrary to the deazaadenine derivatives, the 3-deazaPMEG (*XXIb*) was active against DNA viruses (HSV-1, HSV-2, VZV, CMV)[31]. These results will be published elsewhere.

Because some purine PME derivatives have marked in vitro cytostatic activity on L-1210 mice leukemia cells[13,32], we also studied the inhibitory effect of modified PME derivatives prepared in this study on the growth of L-1210 cell cultures. Compounds *III*, *VII* and *XIIIb* were ineffective up to concentrations 10^{-4} mol l^{-1}. Under the experimental conditions used, the cytostatic effect of 3-deazaPMEG (*XXIb*) was comparable with that of PMEA (*II*) but markedly lower than the cytostatic effect of PMEG (ref.[33]).

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Solvents were evaporated on a rotatory evaporator at 40 °C and 2 kPa. Products were dried over phosphorus pentoxide at 13 Pa.

Thin-layer chromatography was performed on Silufol UV 254 (50 × 16 × 0.3 mm layers), preparative column chromatography on Silpearl UV 254 silica gel (both Kavalier, Votice). The solvent systems are specified in the text. Spots were detected by UV light at 254 nm. Reversed-phase chromatography was performed on octadecyl silica gel (20 μm, Laboratorní přístroje, Praha), detection on a Uvicord 4 701 A (LKB, Sweden) instrument at 254 nm. Preparative HPLC was carried out on an Alltech 300 × 51 mm column packed with Separon SGX-RPS 10 μm; the same type of reversed phase was also used for analytical HPLC (column 200 × 4 mm). The solvent systems used are specified in the text.

Deionization was performed on Dowex 50X8 (H^+ form): after application of the mixture, the column was washed first with water until the UV absorption of the eluate dropped to the original value, and then the compound was eluted with 2.5% aqueous ammonia. In chromatography on Dowex 1X2 (acetate form), the column was first washed with water until the UV absorption of the eluate dropped to the original value and the product was then eluted with a linear gradient of acetic acid or with dilute acetic acid; its concentration for the individual compounds is specified in the text.

Paper electrophoreses were performed on a Whatman No. 3 MM paper at 20 V/cm (1 h) in 0.1 M triethylammonium hydrogen carbonate (TEAB). The electrophoretic mobilities (E_{Up}) are referenced to uridine 3′-phosphate.

UV absorption spectra were measured on a PU 8800 UV-VIS (Pye Unicam) spectrophotometer or on a Beckman DU-65 instrument. Mass spectra were obtained with a ZAB-EQ (VG Analytical) spectrometer, using the EI (electron energy 70 eV) and FAB (ionization by Xe, acceleration voltage 8 kV) techniques.

^1H NMR spectra were measured on a Varian UNITY 200 (200.01 MHz for ^1H) and Varian UNITY 500 (499.8 MHz for ^1H) in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard or in D_2O with sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standard. ^{13}C NMR spectra were measured on a Varian UNITY 200 (50.31 MHz for ^{13}C) spectrometer; the signals were referenced to the solvent

signal, $\delta^{13}C(DMSO) = 39.7$, or to dioxane as external standard; $\delta^{13}C(dioxane) = 66.86$ for solutions in D_2O.

Chemicals and reagents. 1-Deazaadenine (7-amino-3*H*-imidazo[4,5-*b*]pyridine, *IV*) was prepared from 2,3-diaminopyridine using the published methods[16,34–36]. 4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XIV*) was prepared according to the previously described synthesis from tetracyanoethylene[24]. Bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*) was synthesized from 2-chloroethoxymethyl chloride and tris(2-propyl) phosphite as described in ref.[5].

Bromotrimethylsilane and cesium carbonate were Fluka products, 10% palladium on carbon was purchased from Merck, sodium hydride was a Janssen product. Dimethylformamide was dried by distillation from phosphorus pentoxide and stored over molecular sieves.

9-(2-Phosphonomethoxyethyl)-1-deazaadenine (*VII*)

A mixture of 1-deazaadenine (*IV*; 1.00 g, 7.5 mmol), dimethylformamide (30 ml) and 60% sodium hydride dispersion (0.30 g, 7.5 mmol) was stirred at 80 °C for 1 h under exclusion of moisture (calcium chloride tube). Bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*; 2.5 g, 9.6 mmol) was added and the mixture was heated at 100 °C under stirring and exclusion of moisture until the starting base disappeared (5 h) (TLC in chloroform–ethanol 7 : 3). The hot reaction mixture was filtered through Celite which was then washed with dimethylformamide and the filtrate was taken down in vacuo. The residue was codistilled with toluene (2 × 50 ml) and chromatographed on a column of silica gel (100 g) in chloroform. The product *VI* was eluted with chloroform–ethanol (95 : 5). After combining the corresponding fractions and evaporation of the solvents, the residue was dried in vacuo over phosphorus pentoxide; yield 1.25 g (44%) of compound *VI* (amorphous foam).

This product was mixed with acetonitrile (30 ml) and bromotrimethylsilane (3.0 ml) and the mixture was stirred at room temperature for 24 h in a stoppered flask. After evaporation in vacuo, the residue was codistilled with acetonitrile (2 × 50 ml), mixed with water (50 ml), adjusted to pH 8 with triethylamine and allowed to stand for 1 h. The solvent was evaporated in vacuo, the residue deionized on a column of Dowex 50X8 (H^+ form; 100 ml) and then chromatographed on a column of Dowex 1X2 (acetate form; 100 ml). The product was eluted with a linear gradient of acetic acid (0 – 0.5 M, 1 l each). Crystallization from water–ethanol (1 : 4) with addition of ether afforded 0.56 g (59%) of compound *VII*, m.p. >250 °C, $k' = 2.6$ (2% acetonitrile in 0.05 M TEAB), E_{Up} 0.85. For $C_9H_{13}N_4O_4P \cdot H_2O$ (290.2) calculated: 37.24% C, 5.21% H, 19.29% N, 10.68% P; found: 37.77% C, 5.50% H, 19.67% N, 10.50%P. ^1H NMR spectrum (D_2O + NaOD): 8.07 s, 1 H (H-8); 7.75 d, 1 H (H-2, $J(1,2) = 5.4$); 6.42 d, 1 H (H-1); 4.29 t, 2 H (H-1′, $J(1',2') = 4.7$); 3.88 t, 2 H (H-2′); 3.48 d, 2 H (PCH_2, $J(PCH) = 10.1$). ^{13}C NMR spectrum (D_2O): 43.25 s (C-1′); 69.07 d, (PC, $J(P,C) = 150.0$); 70.16 d (C-2′, $J(P,C\text{-}2') = 10.1$); 103.42 s (C-1); 122.23 s (C-5); 142.33 s (C-2); 144.61 s (C-8); 145.89 s (C-4); 146.51 s (C-6). UV spectrum (pH 2): λ_{max} 281.5 nm (ε_{max} 13 500); (pH 13): λ_{max} 262.5 nm (ε_{max} 11 000).

9-(2-Phosphonomethoxyethyl)-3-deazapurine (*XI*)
and 7-(2-Phosphonomethoxyethyl)-3-deazapurine (*XII*)

A mixture of 3-deazapurine (*VIII*; 0.83 g, 7.0 mmol), dimethylformamide (30 ml) and 60% dispersion of sodium hydride (0.28 g, 7.0 mmol) was stirred at 80 °C for 1 h under exclusion of moisture (calcium chloride tube). Bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*; 2.5 g, 9.6 mmol) was added and the mixture was heated at 100 °C for 5 h under stirring and exclusion of moisture. The hot reaction mixture was filtered through Celite which was then washed with dimethylformamide and the filtrate was concentrated in vacuo. The residue was codistilled with toluene (2 × 50 ml) and chromatographed on a column of silica gel (100 g) in chloroform. This chromatography did not separate the diesters *IX* and *X*. The mixture was

dried in vacuo over phosphorus pentoxide; yield 1.0 g (44%) of a mixture of *IX* and *X* as an amorphous foam.

This material was dissolved in acetonitrile (25 ml) and stirred with bromotrimethylsilane (2.5 ml) in a stoppered flask at room temperature for 24 h. The mixture was worked up as described for compound *VII*. The reaction mixture was then deionized on Dowex 50X8 (H^+ form; 100 ml) and the product was chromatographed on a column of Dowex 1X2 (acetate form; 100 ml); elution with a linear gradient of acetic acid (0 – 0.5 M, 1 l each). Crystallization from water–ethanol (1 : 4) with addition of ether afforded 0.35 g (44%) of a mixture of *XI* and *XII* which could not be separated; k = 2.3 (0.05 M TEAB), E_{Up} 0.83. For $C_9H_{12}N_3O_4P \cdot H_2O$ (275.2) calculated: 39.28% C, 5.13% H, 15.26% N, 11.26% P; found: 39.62% C, 5.94% H, 15.18% N, 11.21% P. ^{1}H NMR spectrum (D_2O + NaOD); 7 : 3 mixture of the N^9- and N^7-isomers; N^9-isomer: 8.51 s, 1 H (H-8); 8.83 br s, 1 H (H-6); 8.34 d, 1 H (H-2, J(2,3) = 5.9); 7.84 d, 1 H (H-3); N^7-isomer: 8.56 s, 1 H (H-8); 8.96 br s, 1 H (H-6); 8.28 d, 1 H (H-2, J(2,3) = 5.7); 7.72 d, 1 H (H-3); other protons together: 4.55 t, 2 H (H-1′, J(1′,2′) = 5.0); 4.03 t, 2 H (H-2′); 3.67 d, 2 H (PCH_2, J(P,CH) = 9.1). ^{13}C NMR spectrum (D_2O), N^9-isomer: 45.29 s (C-1′); 67.74 d (PC, J(P,C) = 154.5); 70.42 d (C-2′, J(P,C-2′) = 12.4); 108.22 s (C-3); 137.10 s (C-2); 137.69 s (C-6); 139.00 s (C-5); 141.41 s (C-4); 149.26 s (C-8). ^{13}C NMR spectrum (D_2O), N^7-isomer: 45.8 s (C-1′); 67.74 d (PC, J(P,C) = 154.5); 70.67 d (C-2′, J(P,C-2′) = 12.4); 114.94 s (C-3); 131.54 s (C-5); 131.87 s (C-6); 137.02 s (C-2); 146.51 s (C-4); 150.40 s (C-8).

Bis(2-propyl)-4-amino-6-bromo-5-cyano-1-(2-phosphonomethoxyethyl)pyrrolo[2,3-*d*]pyrimidine (*XV*)

A mixture of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XIV*; 1.2 g, 5.0 mmol), dimethylformamide (15 ml), cesium carbonate (1.0 g; 3.0 mmol) and bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*; 2.0 g, 7.7 mmol) was heated at 100 °C under stirring and exclusion of moisture until the starting base disappeared (24 h; TLC in chloroform–ethanol (8 : 2). After evaporation and codistillation with toluene (2 × 50 ml), the residue was extracted with boiling chloroform (250 ml total), the extract was concentrated and chromatographed on a column of silica gel (100 g) in chloroform. The product *XV* was eluted with chloroform–ethanol (93 : 7). Crystallization from ethanol–ether afforded 1.1 g (48%) of compound *XV*, R_F 0.29 (chloroform–ethanol 9 : 1). For $C_{16}H_{23}BrN_5PO_4$ (460.2) calculated: 41.75% C, 5.04% H, 17.36% Br, 15.21% N, 6.74% P; found: 42.03% C, 4.87% H, 17.73% Br, 14.71% N, 6.59% P. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.34 s, 1 H (H-2); 8.35 and 7.20 2 × br, 2 × 1 H (NH_2); 4.49 t, 2 H (H-1′, J(1′,2′) = 4.9); 3.92 t, 2 H (H-2′); 3.77 d, 2 H (P-CH_2, J(P,CH) = 8.3); 4.44 m, 2 H (POCH, J(P,OCH) = 7.8, J(CH,CH_3) = 6.35); 1.15 and 1.10 2 × d, 2 × 3 H (2 × CH_3). UV spectrum (pH 2): λ_{max} 287.0 nm (ε_{max} 11 800), λ_{max} 259.5 nm (ε_{max} 11 800); (pH 7): λ_{max} 286.0 nm (ε_{max} 11 600), λ_{max} 259.5 nm (ε_{max} 12 500); (pH 13): λ_{max} 287.0 nm (ε_{max} 11 500), λ_{max} 259.5 nm (ε_{max} 12 200).

Bis(2-propyl)-4-amino-5-cyano-1-(2-phosphonomethoxyethyl)pyrrolo[2,3-*d*]pyrimidine (*XIIIa*)

Compound *XV* (1.0 g, 2.2 mmol) was hydrogenated in methanol (50 ml) over 10% palladium on carbon (1.0 g) in the presence of magnesium oxide (1.0 g) at room temperature for 4 h. The reaction mixture was filtered through a layer of Celite, the filtrate was concentrated and the residue purified by preparative TLC in chloroform–ethanol 8 : 2. Crystallization from ethanol afforded 0.42 g (50%) of ester *XIIIa*, R_F 0.18 (chloroform–ethanol 8 : 2). For $C_{16}H_{24}N_5O_4P$ (381.3) calculated: 50.38% C, 6.34% H, 18.36% N, 8.13% P; found: 49.82% C, 6.34% H, 18.01% N, 8.57% P. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.34 s, 1 H (H-2); 7.84 s, 1 H (H-8); 8.20 and 7.00 2 × br, 2 × 1 H (NH_2); 4.54 t, 2 H (H-1′, J(1′,2′) = 4.9); 3.95 t, 2 H (H-2′); 3.76 d, 2 H (PCH_2, J(P,CH) = 8.3); 4.45, 2 H (POCH, J(P,OCH) = 7.6, J(CH,CH_3) = 6.1); 1.15 and 1.10 2 × d, 2 × 3 H (2 × CH_3).

4-Amino-5-cyano-1-(2-phosphonomethoxyethyl)pyrrolo[2,3-*d*]pyrimidine (*XIIIb*)

Bromotrimethylsilane (1.1 ml) was added to a suspension of compound *XIIIa* (0.42 g, 1.1 mmol) in acetonitrile (11 ml). The mixture was stirred in a stoppered flask at room temperature for 24 h, worked up as described in the preparation of compound *VII*, and deionized on Dowex 50X8 (H^+ form; 100 ml). The crude product was chromatographed on a column of Dowex 1X2 (acetate form; 100 ml) and the product was eluted with a linear gradient of acetic acid (0 – 0.5 M, 1 l each). Crystallization from 80% aqueous ethanol with addition of ether afforded 0.25 g (76%) of compound *XIIIb*, m.p. 200 – 202 °C (decomp.), E_{Up} 0.8. For $C_{10}H_{12}N_5O_4P$ (297.2) calculated: 40.41% C, 4.07% H, 23.55% N, 10.43% P; found: 40.24% C, 4.12% H, 23.30% N, 9.82% P. ^{1}H NMR spectrum (D_2O + NaOD): 8.36 s, 1 H (H-2); 7.77 s, 1 H (H-8); 4.55 t, 2 H (H-1′, J(1′,2′) = 5.0); 3.96 t, 2 H (H-2′); 3.47 d, 2 H (PCH_2, J(P,CH) = 8.6). UV spectrum (pH 2): λ_{max} 283.0 nm (ε_{max} 12 000); (pH 13): λ_{max} 277.0 nm (ε_{max} 12 500), λ_{max} 258.0 nm (ε_{max} 13 400).

Bis(2-propyl)-3-(2-phosphonomethoxyethyl)-5-(cyanomethyl)-4-ethoxycarbonylimidazole (*XVIII*)
and Bis(2-propyl)-1-(2-phosphonomethoxyethyl)-5-(cyanomethyl)-4-ethoxycarbonylimidazole (*XIX*)

A stirred mixture of compound *XVII* (1.25 g, 7.0 mmol), dimethylformamide (30 ml), cesium carbonate (1.14 g, 3.5 mmol) and bis(2-propyl) 2-chloroethoxymethylphosphonate (*V*; 2.7 g, 10.5 mmol) was heated at 100 °C under exclusion of moisture until the starting base disappeared (8 h; TLC in chloroform–ethanol 9 : 1). After evaporation of dimethylformamide in vacuo and codistillation with toluene (3 × 50 ml), the residue was extracted with boiling chloroform and chromatographed on a column of silica gel (75 g) in chloroform. The N^3-isomer *XVIII* was eluted with chloroform–ethanol (98 : 2). The corresponding fractions were combined, the solvent evaporated and the residue dried in vacuo over phosphorus pentoxide; yield 2.1 g (76%) of an amorphous foam. ^{1}H NMR spectrum ($(CD_3)_2SO$): 7.89 s, 1 H (–CH=); 4.09 s, 2 H (CH_2); 4.28 q, 2 H (OCH_2CH_3, J(CH_2,CH_3) = 7.1); 1.32 t, 3 H (OCH_2CH_3); 4.45 t, 2 H (NCH_2, J(CH_2,CH_2) = 4.9); 3.80 t, 2 H (OCH_2); 3.75 d, 2 H (PCH_2, J(P,CH) = 8,05); 4.51 m, 2 H (POCH, J(P,OCH) = 7.8, J(CH,CH_3) = 6.35); 1.20 and 1.17 2 × d, 6 H (2 × CH_3).

Further elution with chloroform–ethanol (96 : 4) afforded 0.44 g (16%) of the N^1-isomer *XIX* as an amorphous foam. ^{1}H NMR spectrum ($(CD_3)_2SO$): 7.79 s, 1 H (–CH=); 4.34 s, 2 H (CH_2); 4.26 q, 2 H (OCH_2CH_3, J(CH_2,CH_3) = 7.1); 1.29 t, 3 H (OCH_2CH_3); 4.30 t, 2 H (NCH_2, J(CH_2,CH_2) = 4.6); 3.81 t, 2 H (OCH_2); 3.77 d, 2 H (PCH_2, J(P,CH) = 8.3); 4.52 m, 2 H (POCH, J(P,OCH) = 7.6, J(CH,CH_3 = 6.1); 1.20 and 1.17 2 × d, 6 H (2 × CH_3).

9-(2-Phosphonomethoxyethyl)-3-deazaguanine (*XXIb*)
and 7-(2-Phosphonomethoxyethyl)-3-deazaguanine (*XXb*)

A mixture of crude product *XIX* (0.41 g, 1.1 mmol) or product *XVIII* (0.66 g, 1.6 mmol) and methanolic ammonia (100 ml) was heated in an autoclave at 110 °C for 16 h. After evaporation, the residue was chromatographed on silica gel (50 g) in chloroform. The product *XXIa* or *XXa* was eluted with chloroform–ethanol (92 : 8) to give oily N^9-isomer *XXIa* (0.3 g) or N^7-isomer *XXa* (0.66 g) which was dried over phosphorus pentoxide and then used directly for deblocking.

The N^9- or N^7-isomer obtained above was dissolved in acetonitrile (10 and 15 ml, respectively) and stirred with bromotrimethylsilane (1.0 and 1.5 ml, respectively) in a stoppered flask at room temperature for 24 h. After evaporation and codistillation with acetonitrile (2 × 25 ml), the residue was mixed with water (25 ml). The mixture was kept at pH 8 – 9 for 1 h by addition of triethylamine. The solvent was again evaporated, the residue codistilled with methanol (2 × 50 ml) and deionized on Dowex 50X8 (H^+ form; 50 ml). The crude product was chromatographed on a column of DEAE Sephadex A-25 (acetate form; 50 ml). The column was washed with water until the UV absorption of the eluate dropped to the original value and the product was eluted with a linear gradient of TEAB (0 – 0.3 M, 1 l aech). The UV-

absorbing eluate was concentrated and converted into the free acid by chromatography on a column of Dowex 1X2 (acetate form; 50 ml). The column was washed with water until the UV absorption of the eluate dropped to the original value and then the product was eluted with 1 M acetic acid. After evaporation, the residue was codistilled with water (3 × 20 ml) and crystallized from water–ethanol (1 : 4) with addition of ether. Yield of the N^9-isomer *XXIb* 0.1 g (32% from diester *XIX*), m.p. 250 °C, k = 1.1 (1% acetonitrile in 0.05 M TEAB), E_{Up} 0.88. For $C_9H_{13}N_4O_5P$ (288.2) calculated: 37.51% C, 4.55% H, 19.43% N, 10.76% P; found: 37.79% C, 4.31% H, 19.01% N, 10.53% P. ^{1}H NMR spectrum (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.26 t, 2 H (NCH_2, $J(CH_2,CH_2)$ = 5.4); 3.91 t, 2 H (OCH_2); 3.49 d, 2 H (PCH_2, J(P,CH) = 8.3). ^{13}C NMR spectrum (D_2O): 45.44 s (C-1′); 69.90 d (PC, J(P,C) = 149.4); 71.12 d (C-2′, J(P,C-2′) = 9.5); 92.78 s (C-3); 123.52 s (C-5); 144.44 s (C-4); 145.0 s (C-8); 147.59 s (C-2); 158.68 s (C-6). UV spectrum (pH 2): λ_{max} 313.5 nm (ε_{max} 6 700), λ_{max} 311.5 nm (ε_{max} 6 700), λ_{max} 281.0 nm (ε_{max} 13 400); (pH 7): λ_{max} 273.5 nm (ε_{max} 12 200); (pH 13): λ_{max} 274.5 nm (ε_{max} 19 000).

Yield of the N^7-isomer *XXb* 0.2 g (38% from diester *XVIII*), m.p. 214 – 215 °C, k = 1.92 (2% acetonitrile in 0.05 M TEAB), E_{Up} 0.76. For $C_9H_{13}N_4O_5P$. 2 H_2O (324.2) calculated: 33.34% C, 5.28% H, 17.27% N, 9.56% P; found: 33.70% C, 4.44% H, 17.76% N, 10.34% P. ^{1}H NMR spectrum (D_2O + NaOD): 5.92 s, 1 H (H-3); 8.08 s, 1 H (H-8); 4.54 t, 2 H (NCH_2, $J(CH_2,CH_2)$ = 5.1); 3.93 t, 2 H (OCH_2); 3.48 d, 2 H (PCH_2, J(P,CH) = 8.55). ^{13}C NMR spectrum (D_2O): 47.0 s (C-1′); 69.95 d (PC, J(P,C) = 150.2); 72.10 d (C-2′, J(P,C-2′) = 10.2); 103.60 s (C-3); 114.28 s (C-5); 146.71 s (C-2); 148.0 s (C-8); 153.66 s (C-4); 155.90 s (C-6). UV spectrum (pH 2): λ_{max} 315.5 nm (ε_{max} 7 400), λ_{max} 274.0 nm (ε_{max} 9 000); (pH 7): λ_{max} 314.5 nm (ε_{max} 8 100), λ_{max} 274.0 nm (ε_{max} 9 900); (pH 13): λ_{max} 310.0 nm (ε_{max} 6 700), λ_{max} 261.0 nm (ε_{max} 6 700).

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