SYNTHESIS AND BIOLOGICAL EFFECTS OF 9-(3-HYDROXY-2-PHOSPHONOMETHOXYPROPYL) DERIVATIVES OF DEAZAPURINE BASES

Hana DVOŘÁKOVÁ, Antonín HOLÝ and Petr ALEXANDER

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, The Czech Republic

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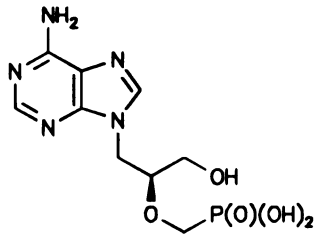
Analogs of antiviral 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine (HPMPA, *I*), containing modified purine bases 3-deazaadenine *XII*, 1-deazaadenine *XIV*, 7-deaza-7-cyanoadenine *XXXII* and 3-deazaguanine *XXXVIII*, were prepared by alkylation of the bases with synthon *XVII*, containing preformed structure of the side chain, in the presence of cesium carbonate. The obtained protected derivatives were deblocked successively with sodium methoxide and bromotrimethylsilane to give phosphonic acids *XII*, *XIV*, *XXXII* and *XXXVIII*. Compounds *XII*, *XIV* and *XVI* were also prepared from (*S*)- or (*R*)-9-(2,3-dihydroxypropyl) derivatives *VI*, *VII* and *XV* by reaction with chloromethanephosphonyl dichloride, isomerization of the arising 2'- and 3'-chloromethanephosphonates and conversion of the 3'-isomers into the phosphonic acids in alkaline medium. The 3-deaza analog *XII* was also prepared by ditritylation of *VI*, reaction with bis(2-propyl) tosyloxymethanephosphonate (*XXII*), subsequent acid hydrolysis and reaction with bromotrimethylsilane. 3-DeazaHPMPA (*XII*) is a potent inhibitor of DNA viruses (HSV-1, HSV-2, VZV, CMV) and exhibits activity against *Plasmodium sp.*

Phosphonomethyl derivatives of acyclic nucleosides (acyclic analogs of nucleotides) represent an extraordinarily biologically attractive group of compounds exhibiting pronounced antiviral activity. The first member of this group whose importance has been recognized is 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine[1] (HPMPA, *I*) which shows high antiviral activity against a whole group of DNA-viruses[2-4].

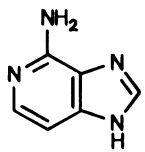
Also other purine HPMP derivatives, particularly those derived from guanine and 2,6-diaminopurine, exhibit high in vitro antiviral activity against herpesviruses (HSV-1, HSV-2, cytomegaloviruses, varicella zoster virus, Epstein–Barr virus, etc.), iridoviruses, adenoviruses and poxviruses[3,5]. The biological activity of the mentioned compounds is not restricted only to the antiviral effects: HPMPA (*I*) specifically influences the growth of broad bean side-roots[6] and is a potent chemosterilant in heteropteras[7].

However, the specificity of the antiviral effect with respect to the base is very limited. Other purine derivatives (e.g. hypoxanthine, xanthine or purine bases substituted in positions 2, 6 and 8) are inactive[3]. A suitable modification of the purine ring, which does not change its spatial demands, is the replacement of the N^1, N^3 or N^7 nitrogen

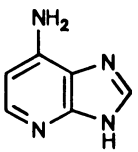
atom by a methine group which gives rise to the so-called deaza derivatives (e.g. 3-deazaadenine (*II*), 1-deazaadenine (*III*), 4-aminopyrrolo[2,3-*d*]pyrimidine (7-deazaadenine, *IVa*) and 3-deazaguanine (*V*)). Such compounds often have significant biological properties (for a review see ref.[8]). For this reason we decided to prepare deaza analogs of HPMPA, HPMPG and related compounds.



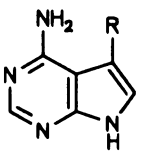
HPMPA (*I*)



II

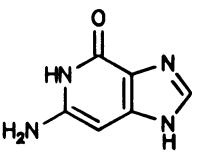


III



IVa, R = H

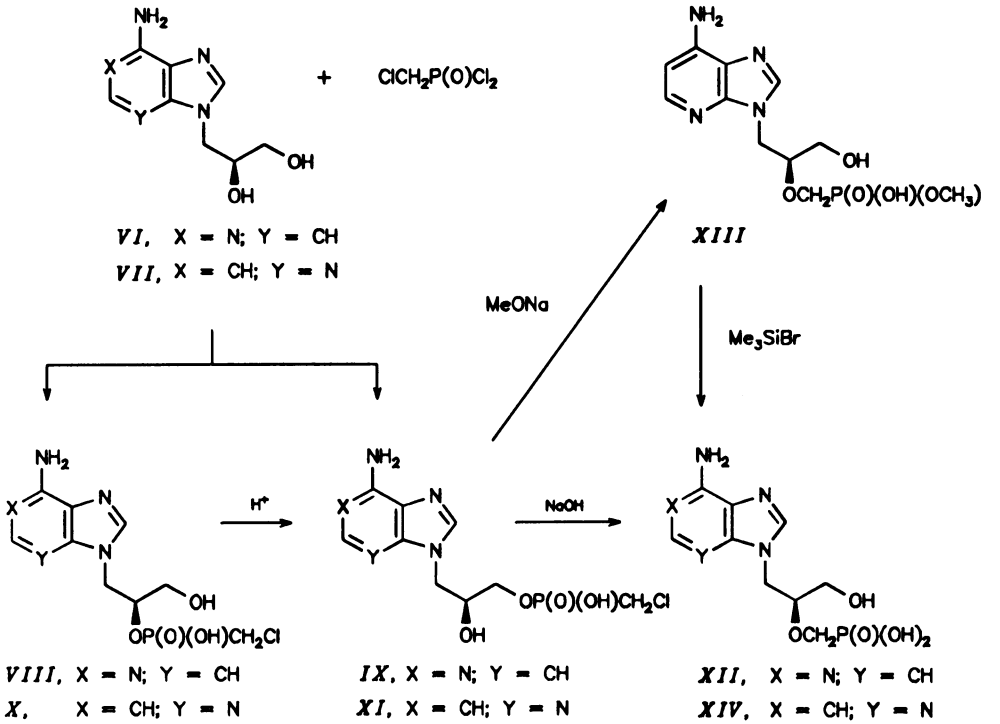
IVb, R = CN



V

In the synthesis of HPMP derivatives, several synthetic paths can be used. For small-scale preparations, the reaction of the already described[9] (*R*)- and (*S*)-9-(2,3-dihydroxypropyl) derivatives of 3-deazaadenine, *VI*, or 1-deazaadenine, *VII*, with chloromethanephosphonyl dichloride in triethyl phosphate[10] (Scheme 1) appeared the method of choice. The 3-deaza analog of HPMPA (3-deazaHPMPA) has already been synthesized by us earlier using this approach[11]. The reaction took place at the vicinal diol grouping of the side chain, affording a mixture of the 2′- and 3′-chloromethanephosphonates *VIII* and *IX* (in the 3-deaza series) or *X* and *XI* (in the 1-deaza series) in practically quantitative yields. The mixture was enriched in the desired 3′-isomer (up to 80%) by boiling in an acid medium and then separated by chromatography on an anion-exchanging resin (Amberlite IRC 50, H^+ form; in the case of 3-deaza analog) or by preparative HPLC on C18 silica gel (1-deaza analog). On heating with aqueous sodium hydroxide solution at 80 °C, the 3′-isomer *IX* afforded crude HPMP derivative *XII*. The 1-deaza derivative required milder conditions: the chloromethanephosphonate *XI* was treated with sodium methoxide to give methyl ester[11] *XIII* which was converted into the

final product *XIV* by reaction with bromotrimethylsilane and subsequent hydrolysis. The crude products were then deionized on a cation-exchanging resin and isolated as free acids *XII* and *XIV* by chromatography on anion-exchanging resin.



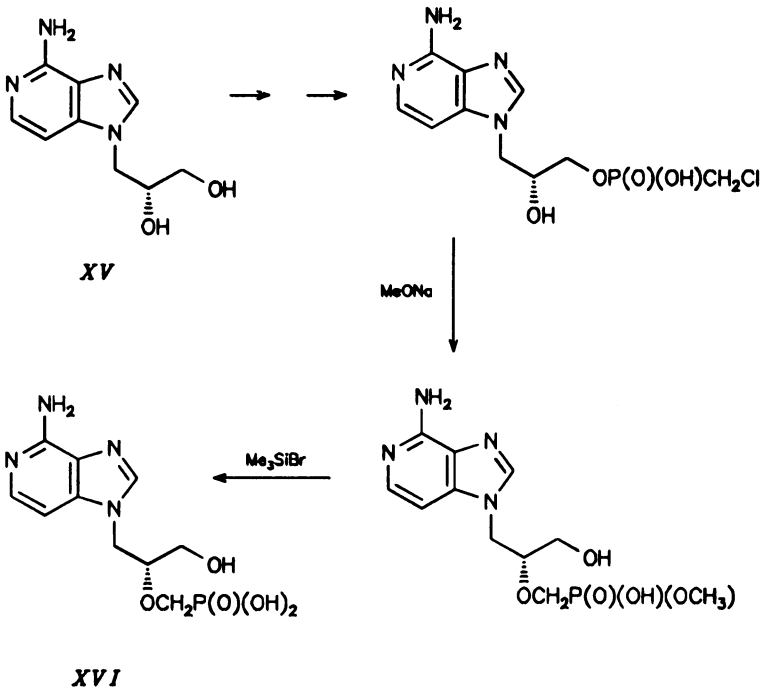
SCHEME 1

The same procedure was also used for the preparation of the (*R*)-enantiomer *XVI* (Scheme 2) from compound *XV*.

Although in many other cases this method was reported to give high yields[11], the yields achieved in the mentioned syntheses were relatively low (about 30% based on the starting 9-(2,3-dihydroxypropyl) derivatives). Another drawback of this procedure consists in the necessary preparation of the optically active 9-(2,3-dihydroxypropyl) derivative.

For this reason we prepared the HPMP derivatives *XII* and *XIV* also by another method, based on alkylation of 3-deazaadenine or 1-deazaadenine with chiral synthon[12] *XVII* containing all the structural elements of the side chain. In our recent communication[9] we described the marked catalytic effect of cesium carbonate on alkylations of

purine bases (and their deaza derivatives) with oxiranes, alkyl halides and alkyl tosylates. This effect also operated in the preparation of HPMP derivatives *XII* and *XIV*. Bases *II* and *III* were alkylated with (*R*)-2-[bis(2-propyl)phosphonomethyl]-3-trimethyl-

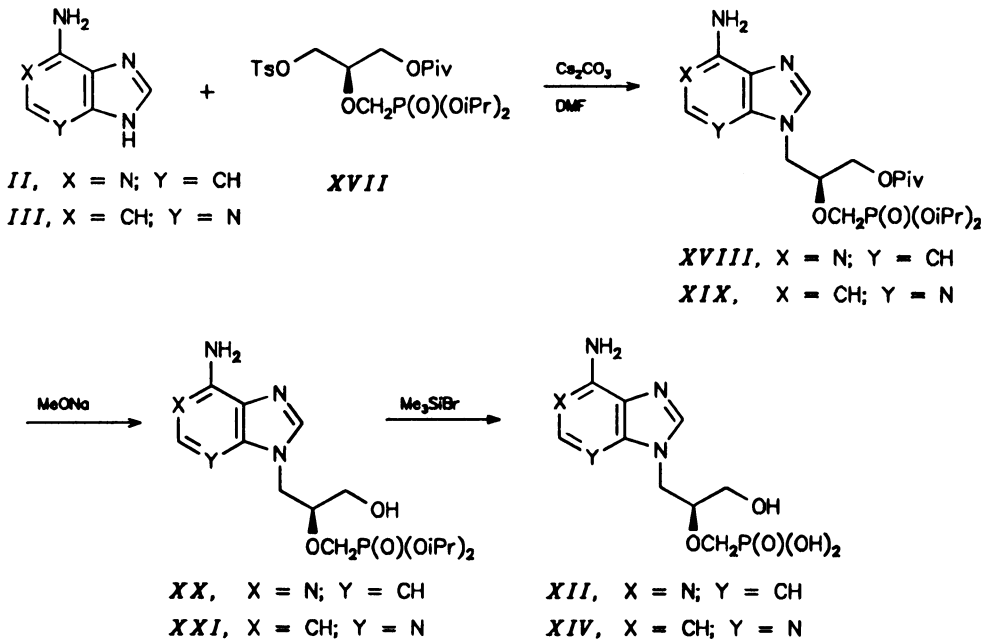


SCHEME 2

acetyl-1,2,3-propanetriol 1-*p*-toluenesulfonate (*XVII*) in the presence of cesium carbonate in dimethylformamide (Scheme 3). The protected intermediates *XVIII* and *XIX* were deblocked by treatment with sodium methoxide and the obtained diesters *XX* and *XXI* were converted into free HPMP derivatives *XII* and *XIV* by cleavage with bromotrimethylsilane and subsequent hydrolysis. Structures of compounds *XII* and *XIV* were assigned on the basis of characteristic maxima in UV spectra which agree with those of the N^9-isomers (e.g. 9-(2,3-dihydroxypropyl) derivatives whose structures were determined by ^{13}C NMR spectroscopy[9]), and comparison with their ^{13}C NMR spectra.

Since, according to preliminary tests, 3-deazaHPMPA (*XII*) had been shown to exhibit a significant antiviral effect, we needed a synthetic method for preparation of larger quantities which would enable studies on experimental animals and which would be economical with respect to the not easily accessible heterocyclic base.

Recently, the optimal synthesis of HPMP compounds on a large scale appears to consist[13] in benzoylation of (*S*)- or (*R*)-(2,3-dihydroxypropyl) derivatives with chlorotrimethylsilane and benzoyl chloride in pyridine[14] and subsequent tritylation to give the protected derivatives with free hydroxyl group in position 2′. In some other cases, the NH_2 group was selectively protected by the amidine functionality (N-dimethylaminomethylene), introduced by reaction with dimethylformamide acetals[15]. The protected trityl derivatives were then condensed in dimethylformamide with one equivalent of bis(2-propyl) ester *XXII* in the presence of excess of sodium hydride. After acid and alkaline deblocking, the isolated diester was converted into the free HPMP derivative by cleavage with bromotrimethylsilane[13].



II, X = N; Y = CH
III, X = CH; Y = N

XVII

XVIII, X = N; Y = CH
XIX, X = CH; Y = N

XX, X = N; Y = CH
XXI, X = CH; Y = N

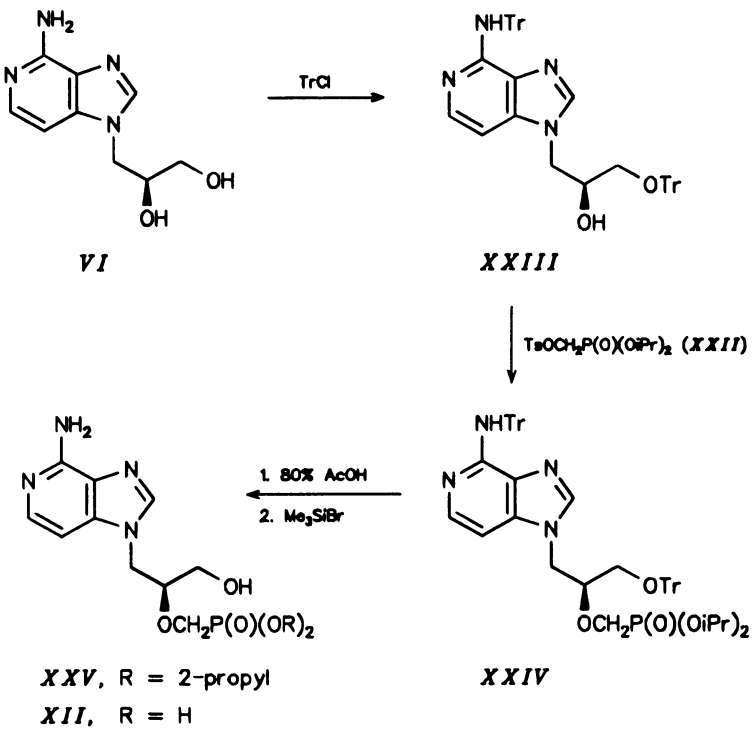
XII, X = N; Y = CH
XIV, X = CH; Y = N

SCHEME 3

In the case of 3-deazaadenine derivatives, the mentioned combinations of protecting groups failed: the N-benzoyl derivatives of this base are extraordinarily stable, obviously thanks to the high basicity of the amino group in position 6. For the same reason, the N-dimethylaminomethylene derivatives are too labile (their lability increases with increasing basicity of the amino group) to be obtained in purity guaranteeing selective tritylation reaction.

An alternative approach to the preparation of HPMPA has also been described that consists in the reaction of N^6,3'-O-dityrityl derivative with the synthon *XXII* in the presence of sodium hydride[16].

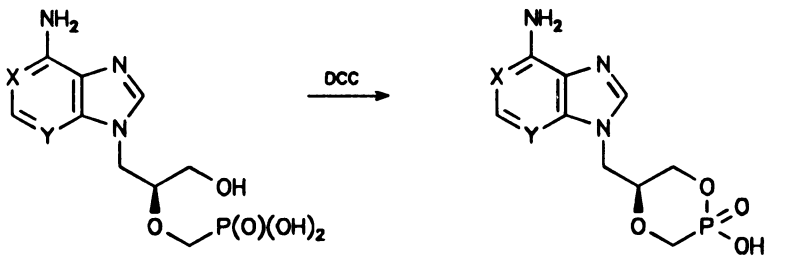
Therefore, we prepared 3-deazaHPMPA (*XII*) by the reaction sequence using N^6,3'-O-ditrityl derivative *XXIII* with the synthon *XXII* (Scheme 4). 3-DeazaDHPA (*VI*) was first tritylated with trityl chloride in dimethylformamide in the presence of N,N-bis-(2-propyl)ethylamine. After separation of the monotrityl derivative by chromatography on silica gel, the ditrityl derivative *XXIII* was condensed with bis(2-propyl) ester *XXII* in the presence of an excess of sodium hydride. After deblocking of the intermediate *XXIV* with 80% acetic acid, the diester *XXV* was converted into the free HPMP derivative *XII* in relatively high yield (41% based on the starting compound *VI*).



SCHEME 4

Some cyclic phosphonates have been reported to exhibit a higher antiviral activity; e.g. the cyclic ester of HPMPA (cHPMPA) has a better selectivity index than HPMPA (*I*) (ref.[3]). This phenomenon is probably due to the enhanced penetration of the cyclic phosphonate into the cells: it is assumed that the cyclic phosphonate acts as a prodrug

which has only one charge and generates the active compound inside the cell under physiological conditions. We therefore also prepared cyclic phosphonates derived from 3- and 1-deazaadenine by cyclization of HPMP compound *XII* or *XIV* using N,N'-dicyclohexylcarbodiimide as activation reagent[17]. The reaction takes place in the presence of morpholino-N,N'-dicyclohexylguanidine at elevated temperature in aqueous tert-butyl alcohol. The cyclic phosphonates *XXVI* and *XXVII* were easily isolated on an anion-exchanging resin (Scheme 5).



XII, X = N; Y = CH
XIV, X = CH; Y = N

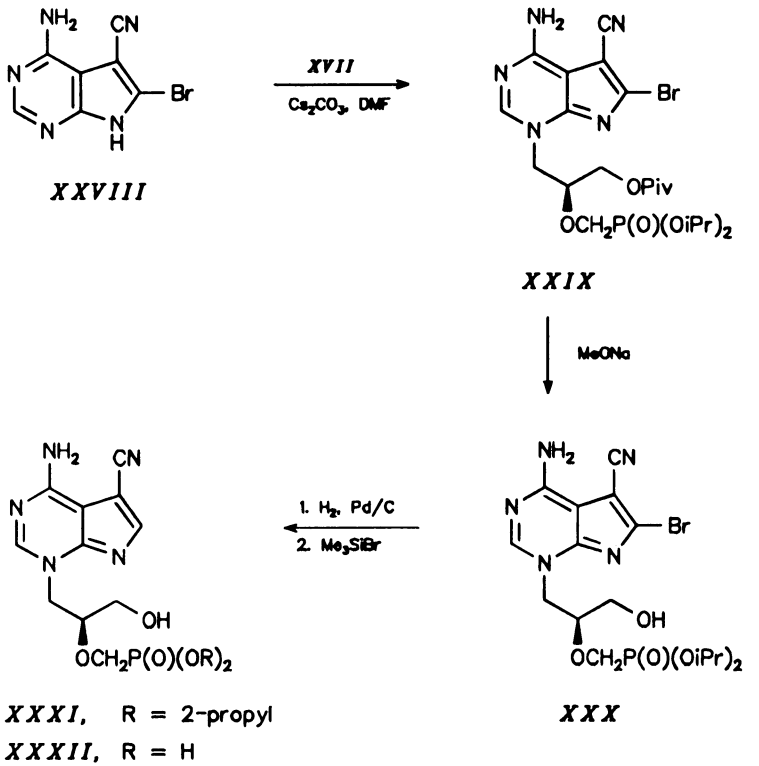
XXVI, X = N; Y = CH
XXVII, X = CH; Y = N

SCHEME 5

Similarly to the 3- and 1-deaza analogs *XII* and *XIV*, we intended to synthesize the HPMP derivative of 7-deazaadenine by alkylation of base *XXVIII* with synthon *XVII* in the presence of cesium carbonate in dimethylformamide. We made use of the known[18] directive effect of bromine in position 6 of the pyrrole ring. However, the regiospecificity of this reaction failed in the case of synthon *XVII*.

Synthon *XVII* was condensed with 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XXVIII*) under formation of the protected bromo derivative *XXIX*. After removal of the pivaloyl group in *XXIX* by methanolysis catalyzed with sodium methoxide, the obtained diester *XXX* was hydrogenated over 10% Pd/C and the crude hydrogenation product was cleaved with bromotrimethylsilane to give free acid *XXXII* (Scheme 6).

It appeared that the 3-deazaguanine analog of HPMPG cannot be prepared by the reaction of the base with the synthon as has been done in all the preceding examples. Such a reaction did not produce detectable quantities of the desired products. This finding is in accord with similar results obtained with the guanine series. Therefore, we made use of the method described by Cook and collaborators[19] for the preparation of 3-deazaguanosine which consists in alkylation of 5(4)-(cyanomethyl)-4(5)-ethoxycarbonylimidazole (*XXXIII*) followed by closure of the pyridine ring by reaction with

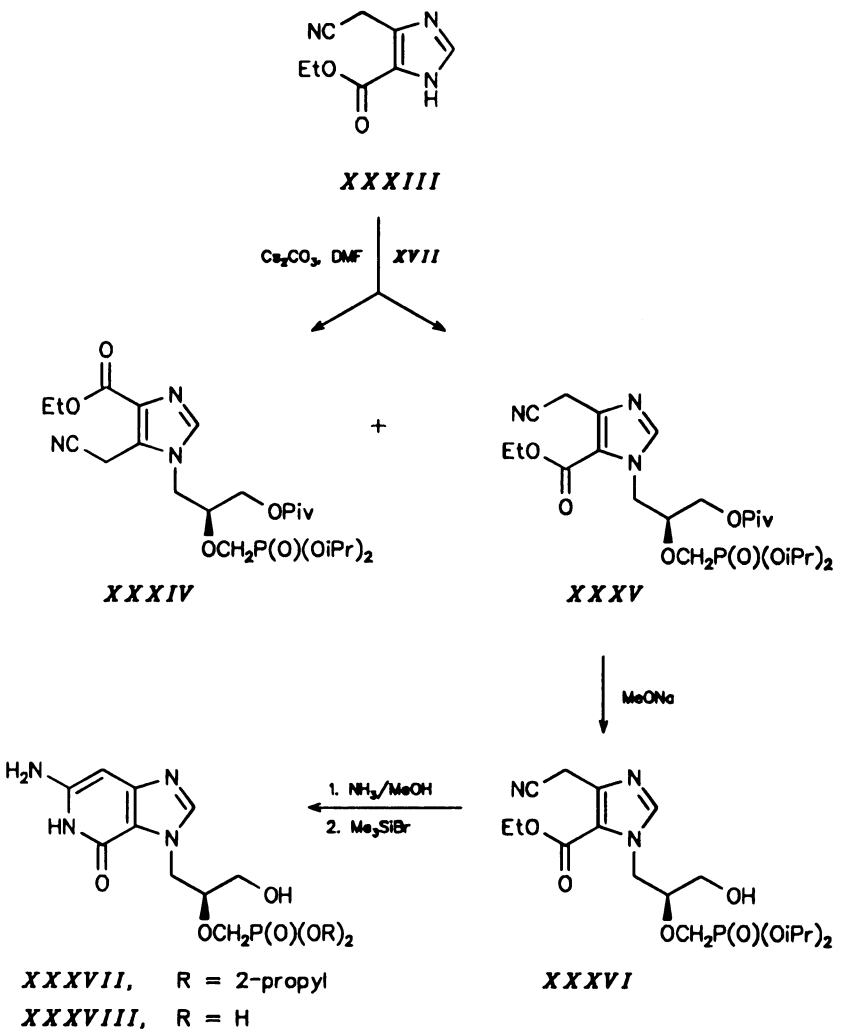


SCHEME 6

methanolic ammonia (Scheme 7). In our case, the synthesis was complicated not only by unstability of the intermediates but also by very similar chromatographic properties of the N^1- and N^3-isomers *XXXIV* and *XXXV*. These facts, supplemented by predominant formation of the N^3-isomer *XXXV* in this alkylation, account for the fact that after cyclization and deblocking we were able to isolate in a very low yield only the N^7-isomer *XXXVIII* whereas the N^9-isomer was not isolated at all. The structure of the N^7-isomer was assigned on the basis of comparison of characteristic UV-maxima with those published in the literature[20].

The antiviral effects of several compounds prepared in this study have already been published[21]. The (*S*)-enantiomer of 3-deazaHPMPA (*XII*) very effectively suppresses the multiplication of herpesviruses (TK^+ and TK^- mutants of HSV-1, HSV-2, VZV and CMV). The absolute values of ED_{50} are lower than those of the parent HPMPA. However, since this enhanced activity is accompanied by higher cytotoxicity, the selectivity index (100 – 500) is somewhat less advantageous than for HPMPA (*I*). As anticipated,

compound *XII* is entirely ineffective against RNA viruses as well as retroviruses. In accord with expectation, also its (*R*)-enantiomer *XVI* is practically devoid of any antiviral effect. By analogy to the adenine series, the cyclic ester of 3-deazaHPMPA (*XXVI*) has better in vitro parameters than 3-deazaHPMPA (*XII*).



SCHEME 7

Both 3-deazaHPMPA (*XII*) and its cyclic ester *XXVI* exhibit a marked effect against African swine fever virus (ASFV), belonging in vitro to the most potent compounds[22] in this respect. Similarly interesting is also the inhibitory effect of 3-deazaHPMPA (*XII*) against *Plasmodium falciparum* and *Plasmodium berghei* (causative agents of malaria); their effect is greater than that of HPMPA (*I*) in a cell culture (ID_{50} = 8 nM and 47 nM, respectively, selectivity index 1 250 and 1 000, respectively). On a model parasitemia in mice, 3-deazaHPMPA (*XII*) was comparably effective as HPMPA (*I*): at 15 mg/kg complete suppression of parasitemia was achieved[23]. Due to this activity, 3-deazaHPMPA (*XII*) represents one of the most interesting compounds of the HPMP series.

The 1-deazaadenine analog of HPMPA *XIV* is much less effective than the mentioned 3-deazaadenine derivative *XII* and its effect concerns exclusively DNA viruses (particularly HSV-1, VZV and CMV), although its ED_{50} values are 10 – 20 times higher than those of 3-deazaHPMPA (*XII*); also its cytotoxicity is lower. Consequently, the selectivity index for both compounds is comparable. A final selection between both compounds can be made only on the basis of more detailed studies on animal models of virus infections.

The other HPMP derivatives prepared in this study, i.e. the 7-cyano-7-deazaadenine derivative *XXXII* and N^7-(3-deazaguanine) derivative *XXXVIII* exhibited no effect against the viruses studied.

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 Czech Republic). 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 given 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.

^{1}H NMR spectra (δ, ppm; *J*, Hz) were measured on a Varian UNITY 200 (200.01 MHz for ^{1}H) and Varian UNITY 500 (499.8 MHz for ^{1}H) 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. 3-Deazaadenine (4-amino-1*H*-imidazo[4,5-*c*]pyridine, *II*) was obtained from 4-aminopyridine by a modified method[9,24,25]. 1-Deazaadenine (7-amino-3*H*-imidazo[4,5-*b*]pyridine, *III*) was prepared analogously, using the described methods[26 - 29]. 4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]-pyrimidine (*XXVIII*) was prepared from tetracyanoethylene according to the previously described synthesis[18].

Bromotrimethylsilane, N,N'-dicyclohexylcarbodiimide and cesium carbonate were Fluka products, 10% palladium on carbon was purchased from Merck, sodium hydride, trityl chloride and N,N-bis(2-propyl)ethylamine from Janssen, chloromethanephosphonyl dichloride from Alfa. Dimethylformamide was dried by distillation from phosphorus pentoxide and stored over molecular sieves.

9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)-1-deazaadenine (*XIV*)

Method A. Chloromethylphosphonyl dichloride (0.2 ml) was added to a stirred solution of compound *VII* (0.27 g, 1.3 mmol) in triethyl phosphate (10 ml) and the mixture was stirred at room temperature overnight until the starting compound disappeared (followed by HPLC; 4% acetonitrile in 0.05 M TEAB). The obtained thick white paste was then diluted with ether (100 ml), the solid was filtered off, washed with ether and dried in vacuo. This mixture, containing predominantly the 2'-isomer (46 : 54), was enriched in the 3'-isomer (70 : 30) by refluxing with water (10 ml) for 24 h (the composition was followed by HPLC; 4% acetonitrile in 0.05 M TEAB; $k_2' = 5.3$, $k_3' = 6.2$). The mixture was deionized on Dowex 50X8 (H^+ form) and further subjected to preparative HPLC in 0.05 M TEAB. The obtained chloromethanephosphonate *XI* (0.34 g, 1.1 mmol) was refluxed with 1 M methanolic sodium methoxide (10 ml) for 6 h, neutralized with Dowex 50X8 (H^+ form; pre-washed with methanol) and made alkaline with triethylamine to pH 9. After removal of the Dowex by filtration, washing with methanol, evaporation of the solvent and codistillation with toluene (3 × 50 ml), the residue was dried over phosphorus pentoxide.

Bromotrimethylsilane (1 ml) was added to a suspension of methyl ester *XIII* (0.34 g, 1.1 mmol) in acetonitrile (10 ml). The mixture was stirred in a stoppered flask at room temperature for 24 h, the solvent was evaporated and the residue was codistilled with acetonitrile (2 × 50 ml). Water (50 ml) was added, the mixture was adjusted to pH 8 with triethylamine and set aside for 1 h. After evaporation, the residue was deionized on Dowex 50X8 (H^+ form; 100 ml) and the solvent was again evaporated. The residue was converted into the sodium salt on Dowex 50X8 (50 ml; Na^+ form); the salt was crystallized from 80% aqueous ethanol, yield 0.16 g (34%), m.p. >250 °C, $k = 3.1$ (2% acetonitrile in 0.05 M TEAB), $E_{Up} = 0.79$. For $C_{10}H_{13}N_4NaO_5P \cdot H_2O$ (364.2) calculated: 15.37% N, 8.51% P; found: 15.41% N, 8.11% P. ^{1}H NMR spectrum (D_2O + NaOD): 8.19 s, 1 H (H-8); 7.94 d, 1 H (H-2, J(1,2) = 5.9); 6.60 d, 1 H (H-1); 4.39 br d, 2 H (H-1, J = 5.5); 3.86 m, 1 H (H-2', ΣJ = 20.0); 3.63 dd, 1 H (H-3', J(3',2') = 3.2, J_g = 12.4); 3.47 dd, 1 H (H-3", J(3",2') = 5.4); 3.54 d, 2 H (J(P,CH) = 9.0, PCH_2). UV spectrum (pH 2): λ_{max} 281.0 nm (ε_{max} 16 600), λ_{max} 260.0 nm (ε_{max} 13 300); (pH 13): λ_{max} 264.0 nm (ε_{max} 14 100), λ_{max} 275.0 nm (ε_{max} 9 400).

Method B. A mixture of 1-deazaadenine (*III*; 1.05 g, 7.5 mmol), 60% sodium hydride dispersion (0.3 g, 7.5 mmol) and dimethylformamide (20 ml) was stirred at 80 °C for 1 h (calcium chloride protecting tube), synthon *XVII* (4.2 g, 8.2 mmol) in dimethylformamide (5 ml) was added and the mixture was heated at 100 °C for 8 h. After evaporation of the solvent and codistillation with toluene (3 × 50 ml), the residue was extracted with boiling chloroform, the extract was filtered and the filtrate concentrated. The residue was chromatographed on silica gel (100 g) in chloroform–ethanol (92 : 8) to give 1.5 g (42%) of oily product *XIX*, R_F 0.6 (chloroform–ethanol 8 : 2) which was directly used in the deblocking step.

A mixture of the obtained compound *XIX* with 0.1 M methanolic sodium methoxide (20 ml) was allowed to stand at 20 °C for 24 h, then neutralized with Dowex 50X8 (H^+ form), the suspension was made alkaline with triethylamine, filtered, the solid was washed with methanol (200 ml) and the filtrate was evaporated; yield 1.0 g of crude bis(2-propyl) ester *XXI* (amorphous foam) which was used directly in the next reaction step.

This material was dissolved in acetonitrile (40 ml), the solution was mixed with bromotrimethylsilane (4 ml) and allowed to stand at 20 °C for 20 h. The mixture was then processed as described in Method *A*. The deionization was performed on Dowex 50X8 (H^+ form; 100 ml). The obtained crude residue after evaporation was applied onto 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 afforded 0.60 g (27%) of compound *XIV*, identical (HPLC, NMR and UV spectrum) with the compound obtained by Method *A*.

9-(*R*)-(3-Hydroxy-2-phosphonomethoxypropyl)-3-deazaadenine (*XVI*)

Chloromethylphosphonyl dichloride (0.5 ml) was added to a solution of compound *XV* (0.62 g, 3.0 mmol) in triethyl phosphate (12 ml) and the mixture was stirred at room temperature overnight. The thick white paste was then diluted with ether (100 ml), the precipitate was filtered off, washed with ether and dried in vacuo. This mixture, containing predominantly the 2'-isomer (75 : 25), was enriched in the 3'-isomer (78 : 22) by reflux in water (14 ml) for 10 h (HPLC in 4% acetonitrile in 0.05 M TEAB, $k_2' = 3.4$, $k_3' = 4.1$). The mixture was deionized on Dowex 50X8 (H^+ form) and further separated on Amberlite IRC50 (H^+ form; 200 – 400 mesh; 500 ml). The column was washed with water and the UV-absorbing fractions (20 ml each) were analyzed by HPLC (4% acetonitrile in 0.05 M TEAB). Fractions, containing the pure 3'-isomer, were combined, neutralized with ammonia and the solvent was evaporated. The obtained chloromethanephosphonate (0.46 g, 1.5 mmol) was refluxed with 1 M methanolic sodium methoxide (15 ml) for 6 h, neutralized with Dowex 50X8 (H^+ form, pre-washed with methanol) and made alkaline with triethylamine to pH 9. The ion exchanger was filtered off, washed with methanol, the filtrate was taken down, the residue was codistilled with toluene (3 × 50 ml) and dried over phosphorus pentoxide.

The thus-obtained methyl ester (0.50 g, 1.5 mmol) was dissolved in acetonitrile (15 ml). Bromotrimethylsilane (1.5 ml) was added and the reaction mixture was stirred in a stoppered flask at room temperature for 24 h. After evaporation, the residue was codistilled with acetonitrile (2 × 50 ml), mixed with water (50 ml) and adjusted to pH 8 with triethylamine. The mixture was allowed to stand for 1 h, then evaporated in vacuo and the residue was deionized on Dowex 50X8 (H^+ form; 100 ml). The obtained crude product was purified by chromatography 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 80% ethanol afforded 0.10 g (11%) of compound *XVI*, m.p. >250 °C, $k = 1.4$ (2% acetonitrile in 0.05 M TEAB), $E_{Up} = 0.78$. For $C_{10}H_{15}N_4O_5P \cdot 3\ H_2O$ (356.2) calculated: 15.72% N, 8.70% P; found: 15.34% N, 8.42% P. ^1H NMR spectrum (D_2O + NaOD): 8.19 s, 1 H (H-8); 7.74 d, (H-2, $J(2,3) = 6.1$); 6.99 d, 1 H (H-3); 4.39 dd, 1 H (H-1', $J(1',2') = 5.1$, $J_g = 14.9$); 4.33 dd, 1 H (H-1'', $J(1'',2') = 6.1$); 3.83 m, 1 H (H-2', $\Sigma J = 20.3$); 3.71 dd, 1 H (H-3', $J(3',2') = 3.7$, $J_g = 12.5$); 3.44 dd, 1 H (H-3'', $J(3'',2') = 5.4$); 3.53 dd, 1 H (J(P,CH) = 8.8, $J_g = 12.5$, PCH_2); 3.47 dd, 1 H (J(P,CH) = 9.5, PCH). ^{13}C NMR spectrum (D_2O): 44.57 s (C-1'); 59.32 s (C-3'); 67.78 d (J(P,C) = 150.2, PC); 79.19 d (J(P,C-2') = 10.9, C-2'); 98.08 s (C-3); 125.56 s (C-5); 137.96 s (C-2); 139.07 s (C-4); 140.79 s (C-8); 150.67 s (C-6). UV spectrum (pH 2): λ_{max} 262.0 nm (ε_{max} 11 600); (pH 13): λ_{max} 264.0 nm (ε_{max} 11 900).

9-(*S*)-(3-Hydroxypropyl-2-phosphonomethoxypropyl)-3-deazaadenine (*XII*)

Method A. A mixture of 3-deazaadenine (*II*; 1.3 g, 10 mmol), 60% sodium hydride dispersion (0.4 g, 10 mmol) and dimethylformamide (35 ml) was stirred at 80 °C for 1 h (calcium chloride protecting tube).

Then, synthon *XVII* (5.6 g, 11 mmol) was added and the mixture was heated at 100 °C for 32 h. After evaporation, the residue was codistilled with toluene (3 × 50 ml), adsorbed on silica gel (10 g) and purified by chromatography on silica gel (50 g) in chloroform–ethanol (48 : 2). Yield 2.2 g (47%) of oily product *XVIII* which was used directly in the next step.

Compound *XVIII* was mixed with 0.1 M methanolic sodium methoxide (20 ml), allowed to stand at 20 °C for 20 h and then neutralized with Dowex 50X8 (H^+ form). The suspension was made alkaline with triethylamine, filtered, the solid was washed with methanol (200 ml) and the filtrate was evaporated to give 1.78 g (4.6 mmol) of compound *XX* as amorphous foam.

The product was dissolved in acetonitrile (50 ml), bromotrimethylsilane (5 ml) was added to this solution, and the mixture was set aside at 20 °C for 20 h. Further work-up was analogous to that described for compound *XVI*. Deionization was performed on Dowex 50X8 (H^+ form, 100 ml). The obtained crude product was applied onto a column of Dowex 1X2 (acetate form; 100 ml) and the product was eluted with 0.5 M acetic acid. Crystallization from 80% aqueous ethanol afforded 0.56 g (41%) of compound *XII*, m.p. >250 °C, k = 1.4, E_{Up} = 0.78, $[\alpha]_D$ −16.8°. For $C_{10}H_{15}N_4O_5P$. 3 H_2O (356.2) calculated: 15.72% N, 8.70% P; found: 15.31% N, 8.75% P. ^1H NMR spectrum (D_2O + NaOD): 8.19 s, 1 H (H-8); 7.75 d (H-2, J(2,3) = 5.7); 7.00 d, 1 H (H-3); 4.36 m, 2 H (NCH_2); 3.50 d, 2 H (J(P,CH) = 9.1, P–CH_2); 3.30 – 3.95 m, 3 H (OCH + OCH_2). ^{13}C NMR spectrum (D_2O): 44.14 s (C-1'); 60.63 s (C-3'); 68.36 d (J(P,C) = 150.9, PC); 80.20 d (J(P,C-2') = 10.3, C-2'); 98.98 s (C-3); 126.15 s (C-5); 139.70 s (C-4); 139.90 s (C-2); 142.50 s (C-8); 151.23 s (C-6). UV spectrum (pH 2): λ_{max} 262.0 nm (ε_{max} 11 600); (pH 13): λ_{max} 264.0 nm (ε_{max} 11 000).

Method B. Sodium hydride (1.9 g, 47.4 mmol, 60% dispersion) was added at −20 °C to a stirred mixture of ditrityl derivative *XXIII* (11.0 g, 15.8 mmol), dimethylformamide (65 ml) and synthon *XXII* (6.65 g, 19 mmol). The mixture was then allowed to warm to room temperature and was stirred under exclusion of moisture until the starting compound disappeared (24 h, monitored by TLC in chloroform–ethanol 9 : 1). The reaction mixture was neutralized with acetic acid (1.9 ml) and the condensation product was directly deblocked by boiling with 80% acetic acid (300 ml) for 30 min. The mixture was taken down, the residue was codistilled with water (3 × 100 ml), mixed with water (500 ml), washed with ether (3 × 200 ml) and deionized on Dowex 50X 8 (H^+ form; 100 ml). The crude product *XXV* (16 mmol) was suspended in acetonitrile (160 ml) and treated with bromotrimethylsilane (16 ml). After stirring in a stoppered flask at room temperature for 24 h, the mixture was worked up as described for compound *XVI*. Deionization was performed on Dowex 50X8 (H^+ form; 200 ml) and the obtained crude residue was applied onto 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) and crystallized from 80% aqueous ethanol with addition of ether; yield 2.95 g (62%) of compound *XII*, identical (HPLC, NMR and UV spectrum) with the compound obtained by Method *A*.

9-(*S*)-(2-Hydroxy-3-triphenylmethoxypropyl)-N^6-triphenylmethyl-3-deazaadenine (*XXIII*)

A mixture of compound *VI* (5.0 g, 24 mmol), dimethylformamide (200 ml), N,N-bis(2-propyl)ethylamine (9.1 g, 72 mmol) and trityl chloride (20.0 g, 72 mmol) was heated at 60 °C for 5 h under stirring and exclusion of moisture. The excess trityl chloride was decomposed by addition of methanol (50 ml) and subsequent stirring for 30 min. After evaporation of the reaction mixture, the residue was codistilled with toluene (3 × 100 ml) and dissolved in chloroform (500 ml). The chloroform extract was washed with water (5 × 100 ml), the solvent was evaporated, the residue was codistilled with toluene and chromatographed on silica gel (200 g) in chloroform. Crystallization from ether–light petroleum afforded 11.0 g (66%) of compound *XXIII*, m.p. 127 – 128 °C, R_F = 0.5 (chloroform–ethanol 9 : 1). For $C_{47}H_{40}N_4O_2$. H_2O (710.8) calculated: 79.41% C, 5.96% H, 7.87% N; found: 79.73% C, 6.14% H, 7.80% N. ^1H NMR spectrum ($(CD_3)_2SO$): 7.98 s, 1 H (H-8); 7.15 – 7.40 m, 31 H (H-2, arom.); 6.68 d, 1 H (H-3, J(3,2) = 5.9); 6.60 s, 1 H (NH); 5.34 d, 1 H (J = 5.6, OH); 4.27 dd, 1 H (H-1', J(1',2') = 3.7, J_g = 14.2); 4.14 dd, 1 H (H-1",

$J(1'',2')$ = 7.6); 3.98 m, 1 H (H-2', ΣJ = 28.0); 3.03 dd, 1 H (H-3', $J(3',2')$ = 5.0, J_g = 9.3); 2.84 dd, 1 H (H-3'', $J(3'',2')$ = 6).

Cyclic Ester of 9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)-3-deazaadenine (*XXVI*) and Cyclic Ester of 9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)-1-deazaadenine (*XXVII*)

A solution of N,N'-dicyclohexylcarbodiimide (1.3 g, 6 mmol) in tert-butyl alcohol (18 ml) was added during 1 h to a stirred and refluxing solution of compound *XII* or *XIV* (0.41 g, 1.16 mmol) and morpholino-N,N'-dicyclohexylguanidine (0.35 g, 1.16 mmol) in 50% aqueous tert-butyl alcohol (36 ml). The mixture was refluxed until the starting compound disappeared (5 h, followed by electrophoresis), the solvent was evaporated, the residue stirred with water and N,N'-dicyclohexylurea removed by filtration through a layer of Celite. The filtrate was concentrated and the residue was deionized on Dowex 50X8 (H^+ form) and chromatographed on a column of Dowex 1X2 (acetate form; 100 ml). The column was washed with water to drop of UV absorption of the eluate to the original value and then the product was eluted with a linear gradient of acetic acid (0 – 0.2 M, 1 l each). The main UV-absorbing fraction was evaporated, the residue codistilled with water and crystallized from 80% aqueous ethanol; yield 0.31 g (94%) of compound *XXVI*, m.p. >250 °C, E_{Up} = 0.47. For $C_{10}H_{13}N_4O_4P \cdot H_2O$ (302.2) calculated: 39.74% C, 5.00% H, 18.53% N, 10.26% P; found: 40.21% C, 5.23% H, 18.25% N, 10.11% P. ^1H NMR spectrum (D_2O + NaOD): 8.02 s, 1 H (H-8); 7.68 d, 1 H (H-2, $J(2,3)$ = 5.8); 6.85 d, 1 H (H-3); 4.24 m, 4 H (NCH_2 and PCH_2); 4.08 m, 1 H (OCH); 3.94 dd, 1 H (H-3', $J(3',2')$ = 8.8, J_g = 14.2); 3.67 dd, 1 H (H-3'', $J(3'',2')$ = 2.4).

Compound *XXVII* was obtained similarly; yield 0.26 g (79%), m.p. >250 °C, E_{Up} = 0.53. For $C_{10}H_{13}N_4O_4P \cdot H_2O$ (302.2) calculated: 39.74% C, 5.00% H, 18.53% N, 10.26% P; found: 39.54% C, 5.55% H, 18.37% N, 10.25% P. ^1H NMR spectrum (D_2O + NaOD): 8.02 s, 1 H (H-8); 7.83 d, 1 H (H-2, $J(2,1)$ = 5.6); 6.50 d, 1 H (H-1); 4.17 – 4.32 m, 4 H (NCH_2 and PCH_2); 4.09 m, 1 H (ΣJ = 23.4, OCH); 3.92 dd, 1 H (H-3', $J(3',2')$ = 8.8, J_g = 14.2); 3.66 dd, 1 H (H-3'', $J(3'',2')$ = 2.4).

4-Amino-5-cyano-1-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)pyrrolo[2,3-*d*]pyrimidine (*XXXII*)

A solution of synthon *XVII* (4.1 g, 8.0 mmol) in dimethylformamide (5 ml) was added to a mixture of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XXVIII*; 1.7 g, 7.0 mmol), dimethylformamide (40 ml) and cesium carbonate (1.1 g, 3.5 mmol). The mixture was heated at 100 °C for 27 h under stirring and exclusion of moisture until the starting base disappeared (TLC in chloroform–ethanol 4 : 1). After evaporation of the solvent, the residue was codistilled with toluene (2 × 50 ml) and extracted several times with boiling chloroform (250 ml total). The extract was concentrated and the residue chromatographed on a column of silica gel (100 g) in chloroform. The condensation product was eluted with chloroform–ethanol (98 : 2), affording 1.8 g (44%) of oily product *XXIX*, R_F = 0.52 (chloroform–ethanol 9 : 1).

The compound *XXIX* was allowed to stand with 0.1 M methanolic sodium methoxide (50 ml) at 20 °C for 24 h and then neutralized with Dowex 50X8 (H^+ form). The suspension was made alkaline with triethylamine, filtered and the resin was washed with methanol (200 ml). Evaporation of the filtrate gave 1.5 g of crude bis(2-propyl) ester *XXX* as an amorphous foam which was used directly in the next reaction step.

A mixture of the crude ester *XXX* (1.5 g, 3.0 mmol), magnesium oxide (1.5 g), 10% Pd/C (1.5 g) and methanol (75 ml) was hydrogenated at room temperature for 3 h and then filtered through a layer of Celite. The filtrate was concentrated and the residue dried over phosphorus pentoxide; yield 1.0 g (80%) of amorphous product *XXXI*, R_F = 0.33 (chloroform–ethanol 9 : 1) which was allowed to stand with acetonitrile (30 ml) and bromotrimethylsilane (3 ml) at 20 °C for 20 h. The mixture was processed as described in the preparation of compound *XVI* and deionized on Dowex 50X8 (H^+ form; 100 ml). The obtained crude residue was chromatographed on a column of Dowex 1X2 (acetate form; 100 ml), the product being eluted with a linear gradient of acetic acid (0 – 0.5 M, 1 l each). Crystallization from 80% aqueous ethanol afforded 0.16 g (7%) of compound *XXXII*, m.p. >250 °C, k = 3.3 (4% acetonitrile in 0.05 M TEAB), E_{Up} =

0.89. For $C_{11}H_{14}N_5O_5P \cdot H_2O$ (345.2) calculated: 38.26% C, 4.67% H, 20.28% N, 8.97% P; found: 38.55% C, 4.37% H, 19.93% N, 8.66% P. ^{1}H NMR spectrum (D_2O + NaOD): 8.34 s, 1 H (H-2); 7.80 s, 1 H (H-8); 4.61 dd, 1 H (H-1', J(1',2') = 3.4, J_g = 14.4); 4.42 dd, 1 H (H-1", J(1",2') = 7.8); 3.90 m, 1 H (H-2', ΣJ = 18.8); 3.86 dd, 1 H (H-3', J(3',2') = 3.4, J_g = 12.2); 3.61 dd, 1 H (H-3", J(3",2') = 4.2); 3.59 dd, 1 H (J(PCH) = 9.8, J_g = 12.45, PCH_2); 3.40 dd, 1 H, J(P,CH) = 9.3 (PCH_2). UV spectrum (pH 2): λ_{max} 285.0 nm (ε_{max} 12 700); (pH 13): λ_{max} 277.0 nm (ε_{max} 13 300), λ_{max} 258.0 nm (ε_{max} 14 100).

7-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)-3-deazaguanine (*XXXVIII*)

A mixture of imidazole *XXXIII* (1.7 g, 9.6 mmol), synthon *XVII* (5.4 g, 10.6 mmol), dimethylformamide (40 ml) and cesium carbonate (1.56 g, 4.8 mmol) was heated at 100 °C for 8 h under stirring and exclusion of moisture until the starting base disappeared (TLC in chloroform–ethanol 9 : 1). After evaporation of the dimethylformamide and codistillation with toluene (3 × 50 ml), the residue was extracted with boiling chloroform and subjected to chromatography. The formed N^1- and N^3-isomers could not be separated even by repeated chromatography on silica gel (80 g) in chloroform. Yield 3.1 g (64%) of a mixture of *XXXIV* (R_F 0.61) and *XXXV* (R_F 0.72). ^{1}H NMR spectrum ($(CD_3)_2SO$): 7.86 s, 1 H and 7.87 s, 1 H (=CH–); 4.10 s, 2 H and 4.11 s, 2 H (CH_2); 4.29 q, 2 H (J = 7.1, OCH_2); 1.32 t, 3 H (OCH_2CH_3); 1.16 s, 9 H and 1.14 s, 9 H (t-Bu); 4.54 m, 2 H (O–CH=); 1.22 d, 6 H and 1.19 d, 6 H (J = 6.1, J = 6.3, CH_3); other signals not distinguished.

In the next step, the mixture of N^1- and N^3-isomers (*XXXIV* and *XXXV*, respectively) was deblocked by treatment with 0.1 M methanolic sodium methoxide (100 ml) for 16 h. The reaction mixture was acidified with Dowex 50X8 (H^+ form), made alkaline with triethylamine, filtered and the resin was washed with methanol. The solvent was evaporated, the mixture was chromatographed on silica gel (80 g) in chloroform and rechromatographed on a preparative thin layer in chlorofom–ethanol (93 : 7), yielding 0.37 g (15%) of compound *XXXVI* which was used directly in the cyclization.

A mixture of the crude product *XXXVI* (0.37 g, 0.89 mmol) and methanolic ammonia (100 ml) was heated at 100 °C for 16 h in an autoclave. After evaporation of the reaction mixture, the residue was chromatographed on silica gel (50 g) in chloroform. The diester *XXXVII* was eluted with chloroform–ethanol (92 : 8); yield 0.3 g of oily crude N^7-isomer *XXXVII* which was dried over phosphorus pentoxide and then used directly in the deblocking step.

A solution of the N^7-isomer in acetonitrile (10 ml) was stirred with bromotrimethylsilane (1 ml) in a stoppered flask at room temperature for 24 h. The mixture was worked up as described for the preparation of compound *XVI* and then deionized on Dowex 50X8 (H^+ form; 50 ml). Separation by preparative HPLC and crystallization from 80% ethanol–water afforded 90 mg (29%) of product *XXXVIII*, m.p. 145 – 149 °C, k = 2.88 (0.05 M TEAB). Mass spectrum (HR, FAB): found (M + H): 319.08; calculated: 318.22. ^{1}H NMR spectrum (D_2O + NaOD): 8.24 s, 1 H (H-8); 4.60 dd, 1 H (H-1', J(1',2') = 3.9, J_g = 14.2); 4.46 dd, 1 H (H-1", J(1",2') = 6.8); 3.86 m, 1 H (H-2', ΣJ = 16.7); 3.83 dd, 1 H (H-3', J(3',2') = 3.4, J_g = 13.2); 3.58 dd, 1 H (H-3", J(3",2') = 4.9); 3.69 dd, 1 H (J(P,CH) = 9.6, J_g = 12.8, PCH_2); 3.49 dd, 1 H, (J(P,CH) = 9.8, PCH_2). UV spectrum (pH 2): λ_{max} 316.0 nm (ε_{max} 6 400), λ_{max} 277.0 nm (ε_{max} 9 300); (pH 7): λ_{max} 315.5 nm (ε_{max} 6 400), λ_{max} 261.5 nm (ε_{max} 5 800); (pH 13): λ_{max} 310.0 nm (ε_{max} 5 200), λ_{max} 260.0 nm (ε_{max} 5 100).

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