SYNTHESIS OF N-(2-(2-PHOSPHONYLETHOXY)ETHYL) DERIVATIVES OF HETEROCYCLIC BASES*

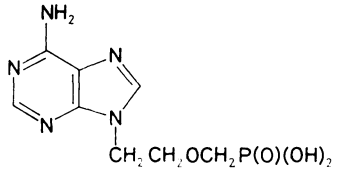
Antonín HOLÝ, Ivan ROSENBERG and Hana DVOŘÁKOVÁ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received June 5, 1989
Accepted June 14, 1989

Reaction of bis(2-chloroethyl) ether (*II*) with triethyl phosphite afforded diethyl 2-chloroethoxyethylphosphonate (*III*). This compound reacts with sodium salts of heterocyclic bases to give diethyl esters of N-(2-(2-phosphonylethoxy)ethyl) derivatives of purine and pyrimidine bases *IV*. Compounds *IV* on reaction with bromotrimethylsilane and subsequent hydrolysis were converted into N-(2-(phosphonylethoxy)ethyl) derivatives *IV*.

In one of our previous communications of this series[1] we have described modifications of the side-chain in phosphonylalkyl ethers of 9-(hydroxyalkyl) adenine derivatives the parent compound of which, 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA), exhibits a high antiviral effect[2-4]. We have found that, like HPMPA, 9-(2-phosphonylmethoxyethyl)adenine (PMEA) (*I*) is also active against DNA-viruses[2]. Other modifications of the side-chain length, its substitution or replacement of the phosphonylmethoxy group in these two parent structures by other phosphonylalkoxy or phosphonyl substituents gave no further compound with antiviral effect[5]. On the other hand, modification of the heterocyclic base in these two structural series[6,7] led to discovery of several new antivirals: derivatives of adenine, guanine, 2,6-diaminopurine and (in the HPMP series) cytosine and 3-deazaadenine exhibit an extraordinary antiviral effect[5]. All the antivirals in both series contain a characteristic phosphonylmethoxy group bonded in the β-position



I

* Part VIII in the series Acyclic Nucleotide Analogues; Part VII: Collect. Czech. Chem. Commun. *54*, 2470 (1989).

of ethyl or hydroxypropyl group. Formally isosteric compounds in which this grouping is replaced by a 4-phosphonylbutyl ("carba-analogs") or 2-phosphonylethoxymethyl group in the same position of the heterocyclic base, were ineffective against any RNA- or DNA-viruses tested[5].

Within the framework of structure–activity studies in the series of acyclic nucleotide analogs we prepared also a homolog of PMEA, 9-(2-(2-phosphonylethoxy)ethyl)-adenine (*VIa*). Preliminary studies of biological properties of this compound have shown that this compound, although without any antiviral effect (vide infra), exhibits a very strong chemosterilizing effect in *Pyrrhococoris apterus* L. (*Heteroptera*)[8]. This finding prompted us to study in detail the preparation of N-(2-(2-phosphonylethoxy)ethyl) derivatives of pyrimidine and purine bases which is the subject of our present communication.

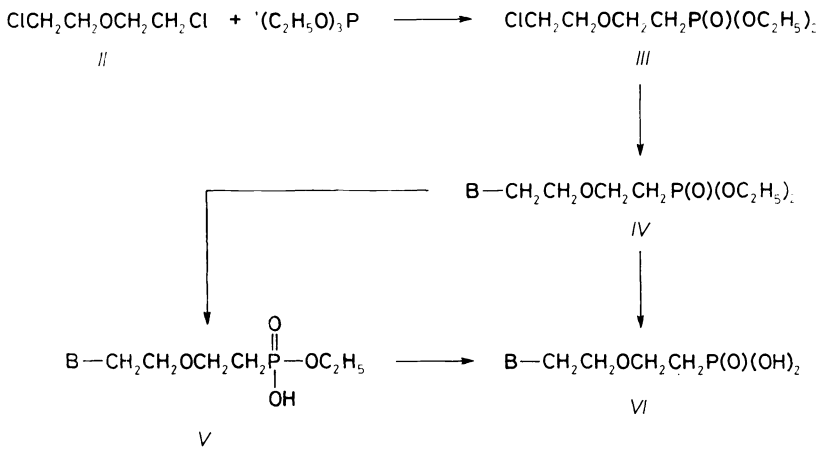
Although it is in principle possible to prepare compounds *VI* by reaction of N-(2-hydroxyethyl) derivatives of heterocyclic bases with dialkyl ethylphosphonate derivatives, suitably activated in position 2, such procedure is not practicable for synthesis of compounds with an identical side-chain but different heterocyclic base. We therefore worked out an approach, starting with a common synthon with preformed side-chain structure. The key compound is diethyl 2-(2-chloroethoxy)ethylphosphonate (*III*), prepared by refluxing triethyl phosphite with bis(2-chloroethyl) ether (*II*) with simultaneous removal of the arising ethyl chloride[9]. The obtained diethyl ester *III* (Scheme 1) was purified by chromatography on silica gel.

This synthon reacted with sodium salts of heterocyclic bases in dimethylformamide under conditions analogous to alkylations with similar organophosphorus synthons[7,10]. The alkylation course depends on the character of the heterocyclic base employed: in some cases (adenine and its derivatives, 6-methylmercaptopurine, N^4-benzoylcytosine) we could use directly the unprotected base and obtained exclusively or predominantly the desired N-9 (in the purine series) or N-1 (in the pyrimidine series) isomers.

The thus-prepared neutral dialkyl phosphonates *IV* were readily separated from the other components of the reaction mixture by chromatography on silica gel and characterized by the UV and NMR spectra. They were converted into phosphonates *VI* by reaction with bromotrimethylsilane and subsequent hydrolysis. Compounds *VI* were purified by chromatography on an anion-exchanger and isolated either as free acids (particularly zwitter-ion forming compounds) or were converted into alkali metal salts.

In cases where the reaction led to stereoisomeric mixtures or the undesired isomer predominated (uracil, guanine or hypoxanthine) we used suitably substituted (protected) bases or their precursors[7]. Thus, for example, with hypoxanthine the alkylation reaction gave the N-7 isomer, guanine and its N-2 acyl derivative afforded a mixture rich in the N-7 isomer; therefore compound *VIh* was prepared by deamination of the adenine derivative *VIa* with 2-methylbutyl nitrite. The prepara-

tion of N-9 substituted guanine derivative *VIf* started from 2-amino-6-chloropurine which was alkylated preferentially in the position N-9 with the synthon *III* in the presence of potassium carbonate. Successive acid and alkaline hydrolysis[7] of the obtained diester *IVd* was accompanied by simultaneous cleavage of the C—Cl and ester bonds under formation of monoester *Vf*. This was isolated by chromatography on an ion-exchanging resin and converted into compound *VIf* by treatment with bromotrimethylsilane (vide supra). The isomeric N-7 derivative *VIe* was prepared in an analogous manner from compound *IVe*, obtained as minor product in the alkylation of 2-amino-6-chloropurine with synthon *III*.



In formulae *IV*–*VI*: *a*, B = adenin-9-yl *b*, B = 6-methylthiopurin-9-yl *c*, B = 2,6-diaminopurin-9-yl *d*, B = 2-amino-6-chloropurin-9-yl *e*, B = 2-amino-6-chloropurin-7-yl *f*, B = guanin-9-yl *g*, B = guanin-7-yl *h*, B = hypoxanthin-9-yl *i*, B = 3-deazaadenin-9-yl *j*, B = 4-methoxy-2-pyrimidon-1-yl *k*, B = uracil-1-yl *l*, B = cytosin-1-yl

SCHEME 1

The uracil derivative *VIk* was prepared by an indirect route starting from 4-methoxy-2-pyrimidone. Sodium salt of this base reacted with synthon *III* to give diethyl ester *IVj* as the principal product, along with some chromatographically readily separable O-2 isomer. Acid hydrolysis of compound *IVj*, followed by alkaline hydrolysis of the ester bond, afforded monoester *Vk* which after isolation was transformed into phosphonate *VIk* by treatment with bromotrimethylsilane and subsequent hydrolysis.

The structure of compounds *VI* follows unequivocally from the structure of the intermediates *IV* and is supported by their characteristic UV spectra. The presence

of the free phosphonate grouping in the molecule of compounds *VI* is confirmed by their electrophoretic mobility which in weakly alkaline medium corresponds to dissociation to the second degree.

None of the prepared N-(2-(2-phosphonylethoxy)ethyl) derivatives *VI* showed any in vitro antiviral effect against vesicular stomatitis virus, herpes simplex virus type 1 and 2, or vaccinia virus* in concentrations up to 400 μg ml^{-1}. The results of further biological tests on these compounds will be published elsewhere.

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Unless stated otherwise, the solvents were evaporated at 40°C/2 kPa and the substances were dried over phosphorus pentoxide at 13 Pa.

Methods

Paper chromatography was performed on a paper Whatman No 1. in 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), (system S1), thin-layer chromatography on Silufol UV 254 plates (Kavalier, Votice, Czechoslovakia) in benzene (S2), chloroform– methanol 9 : 1 (S3), chloroform–methanol 4 : 1 (S4). Paper electrophoresis was carried out on a Whatman No 3. MM paper in 0·05M triethylammonium hydrogen carbonate, pH 7·5 (S5) at 20 V/cm (1 h). UV spectra were measured in aqueous solutions on a PU 8800 UV/VIS spectrophotometer (Pye Unicam, Cambridge, Great Britain), NMR spectra on a Varian XL-200 spectrometer with tetramethylsilane as internal standard; chemical shifts δ are given in ppm, coupling constants J in Hz. Column chromatography was carried out on silica gel according to Pitra (30 μ, Service Laboratories of this Institute) in chloroform or its mixtures with methanol. Mixtures containing compounds with amino groups capable of protonation were desalted on columns of Dowex 50X8 (100—200 mesh) by elution with water until the conductivity and UV absorption of the eluate dropped to the original values and then the products were eluted with 2·5% aqueous ammonia. Chromatography on Dowex 1X2 (100—200 mesh, acetate form) was performed using a linear gradient of acetic acid; in chromatography on Sephadex A-25 (HCO_3^--form) the material was eluted with a linear gradient of the buffer S5. Compounds were detected by continuous measurement of UV absorption of the eluate at 254 nm on a Uvicord instrument (LKB, Uppsala, Sweden). The conversion of free acids or ammonium (or triethylammonium) salts into sodium or lithium salts was performed on column of Dowex 50X8 (100—200 mesh) in the corresponding ion form by elution with water.

Starting Materials and Reagents

Triethyl phosphite, dimethylformamide, sodium hydride, bromotrimethylsilane and adenine were Janssen (Belgium) products, 2,6-diaminopurine and 6-methylmercaptopurine were purchased from Serva (F.R.G.), 2-amino-6-chloropurine from Mack (F.R.G.) and cytosine from Lachema (Czechoslovakia). 4-Methoxy-2-pyrimidone was prepared as described previously[11]. Dimethylformamide and acetonitrile were dried by distillation from phosphorus pentoxide in vacuo and stored over molecular sieves.

* These experiments were carried out at the Rega Instituut, Katholieke Universiteit, Leuven (Belgium) by Professor E. DeClercq.

Diethyl 2-(2-Chloroethoxy)ethylphosphonate (*III*)

A mixture of bis(2-chloroethyl) ether (60 ml, 0·5 mol) and triethyl phosphite (18 ml, 0·1 mol) was heated to 160°C for 10 h, diluted with benzene (100 ml) and the solution was applied onto a column of silica gel (500 g). The material was eluted with benzene–ethanol (99 : 1) and the fractions (à 30 ml) were analyzed by TLC in the system S2 (detection by spraying with *p*-nitrobenzylpyridine followed by exposure to ammonia vapours). Fractions, containing the pure product, were combined, the solvents were evaporated in vacuo and the residue was dried over phosphorus pentoxide in vacuo. The product *III* (12·7 g, 52%) was obtained as a colourless oil. For $C_8H_{18}ClO_4P$ (244·7) calculated: 14·49% Cl, 12·66% P; found: 14·30% Cl, 12·80% P. Mass spectrum, *m/z*: 245 (M + 1), 217 (M − C_2H_3), 209 (M − Cl), 199 (M − C_2H_5O), 195 (M − CH_2Cl), 189 (217 − C_2Ha), 181 (M − C_2H_4Cl). ^{1}H NMR spectrum ($CDCl_3$): 1·33 t, 6 H + 4·11 2 × dq, 4 H ($P(OC_2H_5)_2$, $J(CH_3CH_2)$ = 7·0, $J(POCH)$ = 8·2); 2·13 dt, 2 H (PCH_2, $J(1', 2')$ = 7·4, $J(PH)$ = 18·8); 3·58–3·80 m, 4 H (CH_2CH_2Cl); 3·76 dt, 2 H ($J(1', 2')$ = 7·4, J_{gem} = −12·0).

9-(2-(2-Phosphonylethoxy)ethyl)adenine Diethyl Ester (*IVa*)

A mixture of adenine (13·5 g, 0·1 mol), sodium hydride (obtained from 4·0 g of 60% dispersion in paraffin by washing with light petroleum) and dimethylformamide (700 ml) was stirred at 80°C for 1 h under exclusion of moisture. A solution of compound *III* (22·5 g, 92 mmol) in dimethylformamide (200 ml) was added dropwise under stirring during 2 h and the resulting mixture was stirred under exclusion of moisture at 80°C for another 12 h. The solvent was evaporated at 50°C/2 kPa, the residue was codistilled with toluene (2 × 200 ml) under the same conditions and extracted with boiling chloroform (600 ml). The extract was filtered through Celite which was then washed with boiling chloroform (400 ml) and the filtrate was concentrated in vacuo to about 200 ml. This solution was applied onto a column of silica gel (500 g) which was then washed with chloroform (1 l). Further elution with chloroform–methanol (9 : 1, 30 ml fractions) afforded the product which, after combining the product fractions and evaporation of solvents in vacuo, was crystallized from ether (light petroleum was added to turbidity). Yield 19·5 g (62%) of compound *IVa*, m.p. 60°C, R_F 0·55 (S3). For $C_{13}H_{22}N_5O_4P$ (343·4) calculated: 45·46% C, 6·46% H, 20·40% N, 9·04% P; found: 45·10% C, 6·50% H, 20·25% N, 8·93% P.

9-(2-(2-Phosphonylethoxy)ethyl)adenine (*VIa*)

A mixture of compound *IVa* (19·5 g, 56·8 mmol), acetonitrile (600 ml) and bromotrimethylsilane (38 ml) was stirred to homogeneity and then set aside at room temperature overnight. After evaporation in vacuo, the residue was codistilled with acetonitrile (200 ml) and mixed with 0·4M triethylammonium hydrogen carbonate, pH 7·5 (500 ml). The pH of the mixture was kept between 8–9 for 1 h by addition of triethylamine and then the solution was evaporated in vacuo. The residue was codistilled with methanol (3 × 100 ml), dissolved in water (300 ml) and the solution was applied onto a column of Dowex 50X8 (H^+-form, 500 ml). The column was washed with water to drop of acidic reaction and UV absorption of the eluate to the original values. Then the column was eluted with 2·5% ammonia and the UV-absorbing eluate was concentrated in vacuo. The residue was dissolved in water (100 ml) and applied onto a column of Sephadex A-25 (HCO_3^--form, 400 ml). The column was washed with water to drop of UV absorption of the eluate to the original value and then with linear gradient of 0–0·4M triethylammonium hydrogen carbonate, pH 7·5 (à 2 l; 3 ml/min, fractions à 30 ml). The product-containing fractions were combined and the solvent was evaporated in vacuo. The residue was codistilled with methanol (3 × 100 ml), dissolved in water (100 ml) and applied onto a column of Dowex 50X8

(Na^+-form, 200 ml). The column was eluted with water and the UV-absorbing eluate was evaporated in vacuo. To a solution of the residue in water (20 ml) were added gradually acetone (200 ml) and ether (200 ml). The separated product was collected, washed with acetone–ether (1 : 1), then with ether, and dried in vacuo. Yield 17 g (84%) of disodium salt of compound *VIa*. For $C_9H_{12}N_5O_4PNa_2$ (313·3) calculated: 21·14% N, 9·37% P; found: 20·86% N, 9·12% P. Electrophoretic mobility E_{Up} 0·75 (related to uridine-3′-phosphate); R_F 0·17 (S1). UV spectrum, pH 2: λ_{max} 257·7 nm (ε 11 400).

9-(2-(2-Phosphonylethoxy)ethyl)-6-methylthiopurine (*VIb*)

A mixture of 6-methylthiopurine (3·32 g, 20 mmol), sodium hydride (0·48 g, 20 mmol) and dimethylformamide (80 ml) was stirred at 50°C for 1 h. Compound *III* (6·4 g, 25·2 mmol) was added and the mixture was heated to 100°C for 18 h under exclusion of moisture. The work-up procedure was the same as described for compound *IVa* (the product *VIb* was eluted with chloroform–methanol 95 : 5). The product-containing fractions (R_F 0·68 in S3) were combined and the solvents were evaporated in vacuo. The obtained foam of compound *IVb* (4·0 g, 53%) was dissolved in acetonitrile (80 ml), mixed with bromotrimethylsilane (8 ml) and set aside at room temperature for 48 h. After evaporation of the solvent, the residue was codistilled with acetonitrile (50 ml) and dissolved in 0·4M triethylammonium hydrogen carbonate, pH 7·5 (100 ml). After 30 min the mixture was taken down, the residue was codistilled with methanol (3 × 50 ml), dissolved in water (50 ml) and applied onto a column of Dowex 50X8 (H^+-form, 200 ml). The column was washed with water to drop of the acid reaction of the eluate, and then with 2·5% ammonia. The UV-absorbing eluate was evaporated in vacuo and the residue was dissolved in water (20 ml) and applied onto a column of Dowex 50X8 (Na^+-form, 100 ml). The column was eluted with water and the UV-absorbing eluate was concentrated in vacuo to about 10 ml. To this solution were successively added ethanol (200 ml) and ether (200 ml), the separated product was collected on filter, washed with ether and dried in vacuo; yield 2·8 g (39%) of disodium salt of compound *VIb*, not melting up to 260°C. R_F 0·12 (S1), E_{Up} 0·73. For $C_{10}H_{13}N_4O_4$·.$PSNa_2$ (362·4) calculated: 33·14% C, 3·61% H, 15·47% N, 8·57% P, 8·85% S; found: 33·34% C, 3·75% H, 15·44% N, 8·93% P, 9·07% S.

9-(2-(2-Phosphonylethoxy)ethyl)-2,6-diaminopurine (*VIc*)

A mixture of 2,6-diaminopurine (3·0 g, 20 mmol), sodium hydride (0·48 g, 20 mmol) and dimethylformamide (100 ml) was stirred at 100°C for 1 h. Compound *III* (6·2 g, 25·4 mmol) was added, the mixture was stirred at 100°C for 18 h under exclusion of moisture and then worked up as described for compound *IVa*. Chromatography on silica gel in the system S4 afforded the product *IVc* (4·6 g, 64%) as amorphous foam; R_F 0·40 (S4). A mixture of this compound, acetonitrile (100 ml) and bromotrimethylsilane (10 ml) was allowed to stand for 48 h and processed further as described for compound *VIa*. The ammonia eluate, obtained from the Dowex 50X8 column (deionized crude *VIc*), was dissolved in water (25 ml) which had been adjusted to pH 8·5 with ammonia. The solution was applied onto a column of Dowex 1X2 (acetate form, 200 ml) which was then washed with water to drop of UV absorption of the eluate to the original value. The Dowex was then suspended in 2M formic acid (1 l), the stirred suspension was refluxed for 10 min, filtered while hot and washed with boiling 2M formic acid (1 l). The combined filtrates were evaporated in vacuo, the residue was codistilled with water (5 × 100 ml) in vacuo and dissolved in boiling water. The solution was mixed with a sixfold volume of ethanol and ether was added to persistent turbidity. After standing in a refrigerator, the product was collected, washed with ether and dried in vacuo. Yield 3·0 g (50%) of compound *VIc*, not melting up to

260°C. R_F 0·07 (S1), E_{Up} 0·66. UV spectrum, pH 2: λ_{max} 256, 287·5 nm; λ_{min} 267·5 nm. For $C_9H_{15}N_6O_4P$ (302·3) calculated: 35·75% C, 5·00% H, 27·80% N, 10·27% P; found: 35·46% C, 4·80% H, 27·75% N, 10·34% P.

9-(2-(2-Phosphonylethoxy)ethyl)-2-amino-6-chloropurine Diethyl Ester (*IVd*) and 7-(2-(2-Phosphonylethoxy)ethyl)-2-amino-6-chloropurine Diethyl Ester (*IVe*)

A mixture of 2-amino-6-chloropurine (3·4 g, 20 mmol), potassium carbonate (5·6 g), compound (6·0 g, 24·5 mmol) and dimethylformamide (100 ml) was stirred at 100°C for 30 h and filtered while hot. The precipitate was washed with dimethylformamide (50 ml) and the solvent was evaporated from the filtrate at 40°C/13 Pa. The dry residue was extracted with boiling chloroform (3 × 200 ml), the extract was filtered and the solvent was evaporated in vacuo. The remaining oil was purified by chromatography on a column of silica gel (200 ml), first in chloroform and then in the system S3. The fractions (à 20 ml) were analyzed by chromatography in the system S3 and the product (R_F 0·40) was collected. Evaporation of the solvent and drying in vacuo afforded 3·2 g (42%) of compound *IVd* as a foam. UV spectrum (methanol): λ_{max} 310 nm. Further elution afforded analogously compound *IVe* (1·0 g, 13%) as an amorphous foam, R_F 0·28 (S3). UV spectrum (methanol): λ_{max} 324 nm.

9-(2-(2-Phosphonylethoxy)ethyl)guanine (*VIf*)

A solution of compound *IVd* (3·2 g, 8·4 mmol) in a mixture of dioxane (30 ml) and 1M sodium hydroxide (30 ml) was allowed to stand at room temperature overnight. After neutralization with Dowex 50X8 (H^+-form), the mixture was made alkaline with triethylamine, filtered and the Dowex was washed with water (50 ml). The filtrate was evaporated in vacuo, the dry residue was refluxed with 1M hydrochloric acid (80 ml) for 2 h, made alkaline with ammonia, taken down and dried. The residue was dissolved in water (20 ml) and deionized on a column of Dowex 50X8 (H^+-form, 200 ml) by successive elution with water and dilute aqueous ammonia. The ammonia eluate was evaporated in vacuo, dried by codistillation with ethanol (3 × 50 ml) and over phosphorus pentoxide in vacuo, and suspended in acetonitrile (60 ml). To this supension was added bromotrimethylsilane (6 ml), the mixture was stirred to homogeneity and set aside in a stoppered flask overnight. The solvent was evaporated in vacuo, the residue was codistilled with acetonitrile (50 ml) and dissolved in 0·4M triethylammonium hydrogen carbonate, pH 7·5 (100 ml). After 1 h, the solution was again taken down in vacuo, the residue was codistilled with methanol (2 × 50 ml), dissolved in water (25 ml) and applied onto a column of Sephadex A-25 (HCO_3^--form, 200 ml). The column was washed with water until UV absorption (at 260 nm) of the eluate dropped to the original value and then the product was eluted with a linear gradient (0—0·3M) of triethylammonium hydrogen carbonate, pH 7·5 (à 1 l). The product fraction (0·15—0·25 mol l^{-1}) was taken down in vacuo, the residue was codistilled with methanol (5 × 50 ml) and converted into the sodium salt as described for compound *VIa*. Yield 1·4 g (48%) of compound *VIf*, E_{Up} 0·86, R_F 0·09 (S1). For $C_9H_{12}N_5O_5PNa_2$ (347·3) calculated: 31·12% C, 3·48% H, 20·17% N, 8·94% P; found: 31·30% C, 3·76% H, 19·82% N, 8·79% P.

7-(2-(2-Phosphonylethoxy)ethyl)guanine (*VIg*)

The title compound was prepared from compound *IVe* (1·0 g, 2·6 mmol) as described for compound *VIf*. Yield 0·36 g (38%) of compound *VIg*, E_{Up} 0·86, R_F 0·09 (S1). For $C_9H_{12}N_5O_5PNa_2$ (347·3) calculated: 31·12% C, 3·48% H, 20·17% N, 8·94% P; found: 30·85% C, 3·61% H, 20·06% N, 8·82% P.

9-(2-(2-Phosphonylethoxy)ethyl)hypoxanthine (*VIh*)

3-Methylbutyl nitrite (4 ml) was added to a solution of sodium salt of compound *VIa* (1·0 g, 3·2 mmol) in 80% acetic acid (50 ml) and the mixture was stirred overnight. After evaporation in vacuo, the residue was codistilled with water (5 × 25 ml), dissolved in water (20 ml) and applied on a column of Dowex 50X8 (H^+-form, 150 ml). The material was eluted with water (3 ml/min) and the main UV-absorbing fraction was collected. After evaporation in vacuo, the residue was dissolved in minimum amount of boiling water, mixed successively with tenfold volumes of ethanol and ether. The product, which crystallized on standing in a refrigerator, was collected, washed with ethanol–ether (1 : 1), then with ether, and dried in vacuo. Yield 0·56 g (60%) of compound *VIh*, E_{Up} 0·96, R_F 0·16 (S1). For $C_9H_{13}N_4O_5P$ (288·3) calculated: 37·50% C, 4·54% H, 19·44% N, 10·77% P; found: 37·87% C, 4·85% H, 19·41% N, 11·07% P.

9-(2-(2-Phosphonylethoxy)ethyl)-3-deazaadenine (*VIi*)

A mixture of 3-deazaadenine (1·0 g, 7·5 mmol), dimethylformamide (30 ml) and sodium hydride (0·18 g, 7·5 mmol) was stirred at 80°C for 1 h under exclusion of moisture. Compound *III* (4·2 g, 17·1 mmol) was added and stirring at 80°C was continued for another 30 h under exclusion of moisture. The mixture was filtered through Celite which was then washed with dimethylformamide (20 ml) and the filtrate was evaporated in vacuo. The residue was processed in the same manner as described for compound *IVa*. The product *IVi* (R_F 0·15, S4) was eluted with chloroform–ethanol (4 : 1), the corresponding fractions were combined, the solvents were evaporated in vacuo and the residue was dried in vacuo over phosphorus pentoxide; yield 2·1 g (80%) of compound *IVi* (foam).

This product was dissolved in acetonitrile (10 ml), mixed with bromotrimethylsilane (1·6 ml) and stirred in a stoppered flask at room temperature overnight. After evaporation in vacuo, the residue was codistilled with acetonitrile (3 × 20 ml) and mixed with water (50 ml). The mixture was kept at pH 8—9 for 1 h by addition of triethylamine, water was evaporated, the residue was codistilled with methanol (3 × 20 ml) and dissolved in water (20 ml). The solution was deionized on a column of Dowex 50X8 (H^+-form, 100 ml), the ammonia UV-absorbing eluate was evaporated in vacuo and the residue was dissolved in water (20 ml). The solution was applied onto a column of Dowex 1X2 (acetate form, 100 ml), the column was washed with water until the UV absorption dropped to the original value and the Dowex was transferred into 1M acetic acid (500 ml). After refluxing for 1 h, the hot mixture was filtered, the resin washed with 1M acetic acid (100 ml) and the filtrate evaporated in vacuo. The traces of acetic acid were removed by repeated codistillation with water (3 × 50 ml) and the residue was crystallized from water–ethanol (1 : 4, ether added to turbidity). Yield 0·7 g (41%) of compound *VIi*, not melting up to 260°C. E_{Up} 0·66. For $C_{10}H_{15}N_4O_4P.2\ H_2O$ (322·2) calculated: 17·37% N, 9·62% P; found: 17·01% N, 9·95% P. UV spectrum, pH 2: λ_{max} 261·5 nm (ε_{max} 8 400); pH 12: λ_{max} 265 nm (ε_{max} 8 700). ^{1}H NMR spectrum (D_2O): 1·73 dt, 1 H (H-4′, J(3′, 4′) = 8·5, J(4′, P) = 17·5); 3·72 td, 1 H (H-3′, J(3′, 4′) = 8·5, J(3′, P) = 4·0); 3·88 t, 1 H (H-2′, J(1′, 2′) = 5·0); 4·34 t, 1 H (H-1′, J(1′, 2′) = 5·0); 6·90 d, 1 H (H-3, J(2, 3) = 6·0); 7·72 d, 1 H (H-2, J(2, 3) = 6·0); 8·07 s, 1 H (H-8).

1-(2-(2-Phosphonylethoxy)ethyl)uracil (*VIk*)

Sodium hydride (0·48 g, 20 mmol) was added to a solution of 4-methoxy-2-pyrimidone (2·52 g, 20 mmol) in dimethylformamide (80 ml) and the mixture was stirred at 20°C for 1 h under exclusion of moisture. A solution of compound *III* (6·2 g, 25 mmol) in dimethylformamide (20 ml) was added and the mixture was stirred at 100°C for 20 h (calcium chloride protective tube). After

evaporation at 40°C/13 Pa, the residue was codistilled with toluene (2 × 25 ml) and extracted with boiling chloroform (500 ml total). The extract was evaporated to dryness in vacuo and the residue was subjected to chromatography on a column of silica gel (300 g), first in chloroform and then with chloroform–methanol (49 : 1). Fractions, containing compound *IVj* (R_F 0·70, S3), were combined, the solvent was evaporated in vacuo and the residue was dried over phosphorus pentoxide. The obtained colourless oily product *IVj* was dissolved in acetonitrile (60 ml), mixed with bromotrimethylsilane (6 ml) and the mixture was allowed to stand at room temperature overnight. After evaporation in vacuo, the residue was codistilled with acetonitrile (50 ml) and then dissolved in 0·4M triethylammonium hydrogen carbonate, pH 7·5 (100 ml). After standing for 1 h, the solution was evaporated, the residue codistilled with methanol (3 × 100 ml) and then refluxed with 80% acetic acid (100 ml) for 8 h. The acid was evaporated, the residue codistilled with water (5 × 50 ml) and applied onto a column of Dowex 50X8 (Li^+-form, 100 ml). Elution with water afforded a UV-absorbing fraction which was evaporated in vacuo and the residue was stirred with ethanol (80 ml) for 30 min. Acetone (80 ml) was added, the mixture was stirred for 1 h and the product was filtered, washed with ether and dried in vacuo; yield 1·6 g (29%) of compound *VIk*. E_{Up} 0·96, R_F 0·21 (S1). For $C_8H_{11}N_2O_6PLi_2$ (276·1) calculated: 34·80% C, 4·02% H, 10·15% N, 11·24% P; found: 34·52% C, 4·21% H, 10·06% N, 11·20% P. UV spectrum, pH 2: λ_{max} 260 nm (ε_{max} 10 000).

1-(2-(2-Phosphonylethoxy)ethyl)cytosine (*VII*)

Sodium hydride (0·48 g, 20 mmol) was added to a suspension of N^4-benzoylcytosine (4·3 g, 20 mmol) in dimethylformamide (80 ml) and the mixture was stirred at room temperature for 1 h (calcium chloride protective tube). A solution of compound *III* (6·4 g, 25 mmol) in dimethylformamide (20 ml) was added and the stirred mixture was heated to 100°C for 20 h under exclusion of moisture. After evaporation of the solvent in vacuo and extraction of the residue with chloroform (see the preparation of compound *VIk*), the residue was mixed with 0·1M sodium methoxide in methanol (200 ml) and set aside overnight under exclusion of moisture. The mixture was neutralized by addition of Dowex 50X8 (H^+-form) and made alkaline with triethylamine. The suspension was filtered, the Dowex washed with methanol (200 ml) and the filtrate evaporated in vacuo. The residue was dissolved in water (300 ml) and extracted with ether (3 × 100 ml). The aqueous phase was concentrated in vacuo to half of the original volume and applied onto a column of Dowex 50X8 (H^+-form, 300 ml). The column was washed with water (500 ml) and then with methanol–water (3 : 7) to drop of UV absorption of the eluate to the original value. The product was eluted with 2·5% aqueous ammonia, the UV-absorbing eluate was evaporated in vacuo and the residue was codistilled with ethanol (3 × 50 ml) and dried over phosphorus pentoxide in vacuo. The obtained crude compound *VI* was mixed with acetonitrile (100 ml) and bromotrimethylsilane (10 ml), stirred to homogeneity and set aside for 48 h at room temperature. The mixture was worked up as described for compound *VIk*. The residue, obtained from the decomposed reaction mixture, was deionized on a column of Dowex 50X8 (H^+-form, 200 ml) and the residue after evaporation of the ammonia eluate was chromatographed on a column of Dowex 1X2 (acetate form, 200 ml). The column was washed with water (500 ml) and then with 1M acetic acid. The UV-absorbing fraction, obtained by elution with acetic acid, was evaporated in vacuo, the residue was codistilled with water (4 × 20 ml), dissolved in a minimum amount of boiling water and mixed with a fivefold volume of ethanol. Ether was added to turbidity and the mixture was left in a refrigerator. The product was collected, washed with ether and dried in vacuo; yield 2·0 g (38%) of compound *VII*, m.p. 180°C. E_{Up} 0·85, R_F 0·17 (S1). For $C_8H_{14}N_3O_5P$ (263·3) calculated: 36·49% C, 5·36% H, 15·96% N, 11·79% P; found: 34·46% C, 5·45% H, 15·97% N, 10·57% P. UV spectrum, pH 2: λ_{max} 280 nm (ε_{max} 10 000).

The authors are indebted to Mrs B. Nováková and Mrs B. Česneková of this Institute for excellent technical assistqnce. For carrying out antiviral activity tests they thank Professor E. DeClercq, Rega Instituut, Katholieke Universiteit, Leuven (Belgium). The authors' thanks are also due to Analytical Department of this Institute (Dr V. Pechanec, Head) for carrying out the elemental analyses.

REFERENCES

1. Rosenberg I., Holý A., Masojídková M.: Collect. Czech. Chem. Commun. *53*, 2753 (1988).
2. DeClercq E., Holý A., Rosenberg I., Sakuma T., Balzarini J., Maudgal P. C.: Nature *323*, 464 (1986).
3. Baba M., Mori S., Shigeta S., DeClercq E.: Antimicrob. Agents Chemother. *31*, 337 (1987).
4. DeClercq E., Sakuma T., Baba M., Pauwels R., Balzarini J., Rosenberg I., Holý A.: Antiviral Res. *8*, 261 (1987).
5. Holý A., DeClercq E., Votruba I. in: *Nucleotide Analogs as Antiviral Agents* (J. C. Martin Ed.). ACS Symposium Series, No. 401, p. 51 (1989).
6. Holý A., Rosenberg I., Dvořáková H.: Collect. Czech. Chem. Commun. *54*, 2190 (1989).
7. Holý A., Rosenberg I., Dvořáková H.: Collect. Czech. Chem. Commun. *54*, 2470 (1989).
8. Holý A., Rosenberg I., Gelbič I.: Czech. Appl. PV 5245-88.
9. Marier L., Crutchfield M. M.: Phosphorus Sulfur *5*, 45 (1978).
10. Holý A., Rosenberg I.: Collect. Czech. Chem. Commun. *52*, 2801 (1987).
11. Holý A., Ivanova G. S.: Nucleic Acids Res. *1*, 19 (1974).

Translated by M. Tichý.