SYNTHESIS OF N-(2-PHOSPHONYLMETHOXYETHYL) DERIVATIVES OF HETEROCYCLIC BASES

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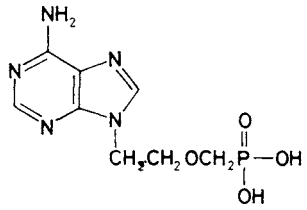
The preparation of N-(2-phosphonylmethoxyethyl) derivatives of purine and pyrimidine bases, *IV*, as analogs of the antiviral 9-(2-phosphonylmethoxyethyl)adenine (PMEA, *I*), is described. The synthesis consists in alkylation of alkali metal salts of heterocyclic bases or their N- or O-substituted derivatives with diethyl 2-*p*-toluenesulfonyloxyethoxymethylphosphonate (*IIa*), 2-chloroethoxymethylphosphonate (*IIb*) or 2-bromoethoxymethylphosphonate (*IIc*). The obtained N-(2-diethoxyphosphonylmethoxyethyl) derivatives of heterocyclic bases (*III*) were treated with bromotrimethylsilane to give phosphonic acids *IV*. Compounds *IV* were prepared from pyrimidines (uracil, cytosine and their 5-methyl derivatives), purines (adenine and its N^6- and C(2)-substituted derivatives, hypoxanthine, guanine, 6-hydrazinopurine and 6-methylthiopurine etc.) and their analogs (3-deazaadenine etc.).

In one of our recent communications of this series[1] we have described the methods for preparation of 9-(2-phosphonylmethoxyethyl)adenine (PMEA, *I*). This acyclic nucleotide analog exhibits interesting antiviral activity against some DNA viruses (e.g. HSV-1 and HSV-2) (refs[2,3]) and retroviruses (MSV, HIV) (refs[4,5]). Within the framework of our structure–activity studies we investigated its analogs obtained by extensive side-chain modifications of the parent structure *I*, which are derived from



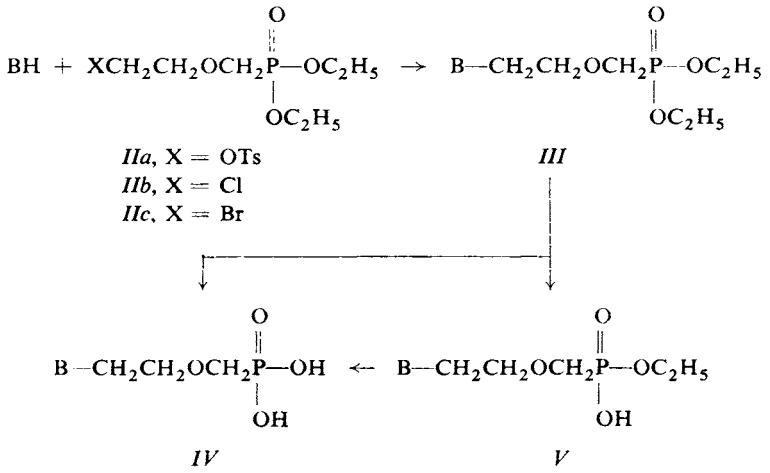
I

the 9-substituted adenine system[6,7]. In the present communication we describe the preparation of such analogs of compound *I* in which the side-chain structure is preserved whereas the adenine residue is replaced by other heterocyclic bases. i.e.

Part VI of the series Acyclic Nucleotide Analogues: Part V: Collect. Czech. Chem. Commun. *54*, 446 (1989).

the synthesis of N-(2-phosphonylmethoxyethyl) derivatives of pyrimidine and purine bases.

The hitherto used syntheses of compound *I* were based either on substitution of 9-(2-hydroxyethyl)adenine or on alkylation of adenine with an organophosphate synthon with preformed structure of the side-chain. For solution of the given task the second approach is logically more convenient. A general synthetic pathway leading to the N-(2-phosphonylmethoxyethyl) derivatives *IV* is depicted in Scheme 1.



B = pyrimidin-1-yl, purin-9-yl, Ts = *p*-toluenesulfonyl residue

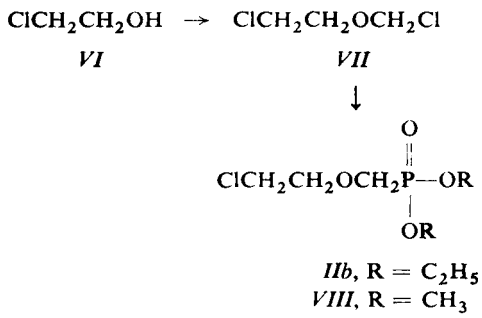
SCHEME 1

The heterocyclic base BH reacts with the organophosphorus synthon *II* to give intermediary dialkyl phosphonate *III*. Hydrolysis of the ester functionalities leads then to the desired compound *IV*.

Any compound of the formula *II*, described in our previous communication[1], may serve as the synthon in the above mentioned reaction. The chloro derivative *IIb*, prepared previously from diethyl 2-hydroxyethoxymethylphosphonate by treatment with triphenylphosphane and tetrachloromethane[1], has been now obtained by a more advantageous procedure[8], described in Scheme 2.

Reaction of 2-chloroethanol (*VI*) with 1,3,5-trioxane and hydrogen chloride affords 2-chloroethoxymethyl chloride (*VII*) which by Arbuzov reaction with triethyl phosphite is converted into the chloro derivative *IIb* in high yield. Thanks to the large difference in reactivity of both the C—Cl bonds in compound *VII*, the reaction course is unequivocal. The procedure requires no chromatographic purification of compound *IIb* from side-products of the reaction with triphenylphosphane, pure product

being obtained by simple vacuum distillation. This new method of preparation makes the compound *IIb* a more accessible and suitable reagent for the synthesis of phosphonyl derivatives *IV*, particularly for larger-scale syntheses. The dimethyl ester *VIII* may be prepared by reaction of chloromethyl ether *VII* with trimethyl phosphite in the same manner as the compound *IIb*.

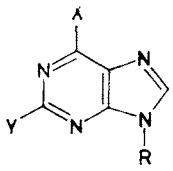


SCHEME 2

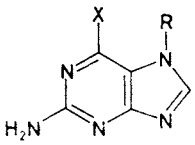
The alkylation was performed in dimethylformamide because of its excellent solvation properties and its directive effect on the course of alkylation. In all cases the synthon *II* reacted with an alkali metal salt of the heterocyclic base, generated in situ by treatment with sodium hydride; in the case of *IIb* the reaction was also carried out in the presence of potassium carbonate.

When the alkylation with synthon *IIb* leads to the desired N-alkyl isomer, the starting heterocyclic base can be used in its unprotected form. This is the case with adenine, its N^6,C(2)-substituted derivatives, 6-methylpurine, 6-methylthiopurine and in part also with 2-aminopurine[9] which afford predominantly N^9-substituted derivatives, with 4-aminopyrazolo[5,6*d*]pyrimidine (N^7-isomer), with cytosine (which gives an N^1-alkylated product), etc. If, however, the reaction of the free base leads to a mixture of N-isomers or to the undesired isomer, a suitably protected derivative has to be used. Such situation arises e.g. with uracil, thymine (formation of a mixture of N^1- and N^1,N^3-disubstituted derivatives), hypoxanthine (N^7-isomer) or guanine (a mixture of N^7- and N^9-isomers).

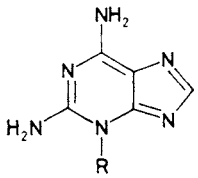
The first two bases may be employed in the form of their 4-O-alkyl derivatives (substituted 4-methoxy-2-pyrimidones) and the conversion into the uracil or thymine derivative may then be effected by acid hydrolysis in some of the further synthetic steps. The reaction of synthon *II* with sodium salt of uracil leads predominantly to the N^1-isomer *IIIq*, affording simultaneously minor amounts of the N^1,N^3-disubstituted derivative which was characterized as the tetraester *XIa* and as the diacid *XIb* (after removal of the ester groups).



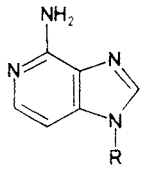
a, X = Y = NH_2 *h*, X = CH_3
d, X = OH ; Y = H *j*, X = Cl ; Y = NH_2
e, X = H ; Y = NH_2 *l*, X = OH ; Y = NH_2
f, X = SCH_3 ; Y = H *m*, X = OH ; Y = $NHCOCH_3$
g, X = $NHNH_2$



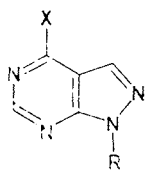
b, X = NH_2
i, X = Cl
k, X = OH



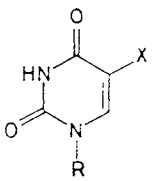
c



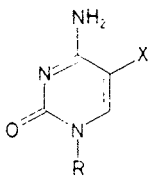
p



n, X = NH_2
o, X = CH



q, X = H
r, X = CH_3



s, X = H
t, X = CH_3

In formulae *III*, R represents $CH_2CH_2OCH_2P(O)(OC_2H_5)_2$;
in formulae *IV*, R represents $CH_2CH_2OCH_2P(O)(OH)_2$;
in formulae *V*, R represents $CH_2CH_2OCH_2P(O)(OC_2H_5)(OH)$

In the preparation of the N^9-isomer derived from hypoxanthine and its derivatives, deamination of adenine derivatives (such as compound *I*) is the method of choice. With guanine (or N^2-acetylguanine), the overall conversion is very low; the best route consists in reaction of 2-amino-6-chloropurine (the alkylation gives both isomers in the ratio 8 : 1 in favour of the N^9-alkyl derivative *III*) and acid hydrolysis[10] of the obtained intermediate to the guanine derivative. Low solubility of some free

bases (guanine, cytosine and its derivatives) in the reaction medium may also represent a considerable limitation. In such cases one may employ suitably substituted derivatives, such as N^4-benzoylcytosine; no loss of the benzoyl groups takes place when the reaction is performed with sodium hydride at low (or room) temperature.

Isolation of the neutral diesters *III* (Table I) is the necessary condition for preparation of isomerically pure compounds *IV*. After extraction with chloroform from the reaction mixture, the intermediates *III* can be easily purified by chromatography on silica gel or directly by crystallization. This procedure is necessary even when the reaction leads predominantly to one isomer. By performing the reaction on a larger scale and isolating the minor side-products, one can obtain the isomers of compounds

TABLE I
Characteristics of compounds *III*

Compound	M.p. °C	R_F		Formula (M.w.)	Calculated/Found			
		S1	S2		% C	% H	% N	% P
IIIa	>250	—	0·53	$C_{12}H_{21}N_6O_4P.$ $.C_2H_5OH$ (390·5)	43·06 43·15	6·97 6·84	21·53 21·55	7·95 8·14
IIIe	—	—	0·44	—	—	—	—	—
IIIf	—	0·60		—	—	—	—	—
IIIg	—	—	0·78	—	—	—	—	—
IIIh	—	0·34	—	—	—	—	—	—
IIIi	133	0·33	—	$C_{12}H_{19}ClN_5O_4P$[a] (363·8)	39·61 39·68	5·27 5·30	19·25 19·15	8·53 8·53
IIIj	111	0·43	—	$C_{12}H_{19}ClN_5O_4P$[b] (363·8)	39·61 39·71	5·27 5·50	19·25 19·17	8·53 8·23
IIIm	157	—	0·50	$C_{14}H_{22}N_5O_6P$ (387·4)	43·40 43·38	5·72 5·91	18·08 18·26	8·01 7·89
IIIn	94—96	0·40	—	$C_{12}H_{20}N_5O_4P$ (329·4)	43·76 44·06	6·12 5·95	21·27 21·06	9·42 9·27
IIIq	—	0·38	—	$C_{11}H_{19}N_2O_6P$ (306·3)	43·12 43·23	6·25 6·52	9·15 9·27	10·13 9·84
XIa	—	0·48	—	$C_{18}H_{34}N_2O_{10}P_2$ (500·6)	43·19 42·91	6·85 6·77	5·60 5·88	12·40 12·21

[a] Calculated: 9·75% Cl; found: 9·69% Cl; [b] calculated: 9·75% Cl; found: 10·02% Cl.

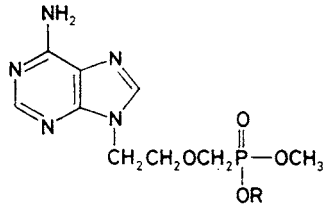
IV that are otherwise accessible only with difficulty. We thus isolated e.g. the N^7- and N^3-isomers derived from 2,6-diaminopurine (*IVb* and *IVc*).

The diesters *III* were cleaved using the previously described[1] reaction with bromotrimethylsilane in acetonitrile*. After removal of excess reagent, hydrolysis of the reaction mixture and deionization, the N-(2-phosphonylmethoxyethyl) derivatives *IV* are purified by chromatography on anion exchangers. In most cases it is possible to use a strongly basic anion exchanger in the acetate form and to isolate the product *IV* as the free acid, often crystallizable from water or aqueous ethanol as a hydrate. However, the 2,6-diaminopurine and guanine derivatives (*IVa* and *IVb*, respectively) are so sparingly soluble in water that the ion-exchanger chromatography has to be carried out in a mildly alkaline medium on a medium basic anion-exchanger (e.g. Sephadex A-25).

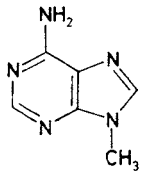
Also the two-stage alternative may be used in the preparation of compounds *IV*: in the first step one ester group is saponified in an alkaline medium under formation of monoesters of phosphonyl derivatives *V* (Scheme 1). The second ester functionality is completely acid- and alkali-stable, allowing thus the desired transformations on the heterocyclic base (e.g. methanolysis of the N-benzoyl group or hydrolysis of the C—Cl bond in 2-amino-6-chloropurine derivatives *IIIi* or *IIIj*). The monoesters *V* may be either isolated by ion-exchanger chromatography or, without isolation, they can be directly converted into compounds *IV* by reaction with bromotrimethylsilane.

We also studied the possible use of dimethylester *VIII* in the preparation of compounds *IV*. In a model reaction (alkylation of sodium salt of adenine under usual conditions) the expected diester *IXa* indeed arose but was cleaved to give the monomethyl ester *IXb* already during the alkylation. In addition to the low yield of compound *IXa* and work-up complications due to the mentioned cleavage, the presence of significant amount of 9-methyladenine (*X*) in the reaction mixture represents another drawback. Compound *X* (characterized by the ^{13}C and ^1H NMR spectra and comparison with an authentic sample) is evidently the product of methylation of adenine with dimethyl phosphonate *VIII* which proceeds concurrently with the desired alkoxyethylation. Analogous reactions are known for trimethyl phosphate and phosphite. We cannot exclude that also the dimethyl ester *IXa* might possess such methylation ability: this would explain the significant amount of monomethyl ester *IXb* in the reaction mixture. Therefore, the best alternative for preparation of compounds *III* and *IV* appears to be working with diethyl esters *II* (the amount of N^9-ethyl derivatives as the reaction side-products, though detectable, is negligible), or such esters of phosphonic acids (e.g. 2-propyl, 2,2-dimethylpropyl) in which the alkylation reaction is a priori excluded.

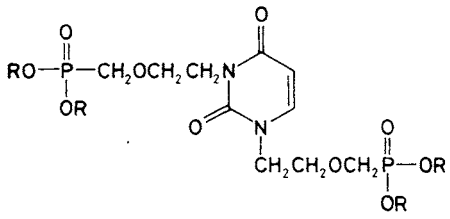
* 4-O-Methyl derivatives of the pyrimidine series partly lose the O-methyl group in the reaction with bromotrimethylsilane; on the other hand, a methylthio group in position 6 of the purine system (compound *IVf*) is stable under the same reaction conditions.



IX a, R = CH_3
IX b, R = H



X



XI a, R = C_2H_5
XI b, R = H

Products *IV* were characterized by usual analytical methods, including elemental analysis and UV and NMR spectra. Purity of the compounds was checked by HPLC, paper chromatography and electrophoresis in mildly alkaline medium (under these conditions the mobility of compounds *IV* corresponds to dissociation to the second degree (Table II)). In most cases their structure followed already from that of the diesters *III*, confirmed by the NMR spectroscopy. Using this method, the isomeric side-products of the reaction of compound *IIc* with 2,6-diaminopurine were characterized as the ethyl esters *Vb* and *Vc* and as the phosphonates *IVb* and *IVc*. Also the UV spectra were employed for the structural assignments: by comparison with the known data it was possible to distinguish the N^1- and N^3-isomers in the pyrimidine series, the N^7- and N^9-alkyl derivatives of guanine, 2-aminopurine etc.

Although the phosphonyl derivatives *IV* can be isolated as free acids, these forms are of little use for biological purposes and they were therefore converted into their sodium salts which are well soluble in water. The transformation was realized either by neutralization of the free acids or by an ion exchange from ammonium or triethylammonium salts. According to our experience, an additional ion exchange is desirable even when the sodium salt is prepared by neutralization of the acid *IV* because the thus-obtained sodium salt can be well precipitated with ethanol or acetone from concentrated aqueous solutions.

The phosphonic acids of the general formula *IV*, their monoethyl esters *V* and other derivatives prepared in this study, were subjected to antiviral activity assays and

TABLE II

Characteristics of compounds *IV*

Compound	R_F (S3)	E_{Up} (S4)	k (S)[a]	Formula (M.w.)	Calculated/Found			
					% C	% H	% N	% P
I	0·18	0·86	2·52 (S6)	—	—	—	—	—
IVa	0·08	0·84	4·74 (S5)	$C_8H_{13}N_6O_4P$ (288·3)	33·33 33·11	4·54 4·70	29·16 28·81	10·77 10·60
IVb	0·08	0·82	5·45 (S5)	$C_8H_{11}N_6O_4PNa_2$ (332·3)	— —	— —	25·30 24·93	9·34 9·07
IVc	0·14	0·84	3·52 (S5)	$C_8H_{11}N_6O_4PNa_2$ (332·3)	— —	— —	25·30 24·79	9·34 9·02
IVd	0·17	0·90	0·74 (S6)	$C_8H_{11}N_4O_5P$ (274·3)	35·03 35·54	4·04 4·31	20·43 20·42	11·32 11·84
IVe	0·16	0·84	1·56 (S6)	$C_8H_{10}N_5O_4PNa_2.H_2O$ (335·3)	— —	— —	20·89 20·88	9·26 9·47
IVf	0·37	0·90	3·29 (S7)	$C_9H_{11}N_4O_4PLi_2S$[b] (316·2)	34·18 34·12	3·51 3·26	17·72 17·58	9·82 10·01
IVg	0·20	0·96	1·84 (S6)	$C_8H_{11}N_6O_4PNa_2$ (332·3)	— —	— —	25·30 25·04	9·34 9·58
IVh	0·26	0·84	7·20 (S6)	$C_9H_{11}N_4O_4PNa_2.2H_2O$ (352·3)	— —	— —	15·90 16·03	8·81 8·85
IVk	0·06	0·94	3·38 (S4)	$C_8H_{10}N_5O_5PNa_2.2H_2O$ (369·3)	26·02 25·97	3·82 3·69	18·96 18·57	8·40 8·30
IVl	0·06	0·88	1·00 (S6)	$C_8H_{10}N_5O_5PNa_2.H_2O$ (351·3)	— —	— —	19·94 19·87	8·84 9·00
IVn	0·21	0·78	6·05 (S6)	$C_8H_{10}N_5O_4PNa_2$ (317·3)	— —	— —	22·08 21·81	9·78 9·71
IVo	0·18	0·82	1·54 (S6)	$C_8H_{11}N_4O_5P$[c] (274·3)	35·03 34·92	4·04 4·26	20·43 20·60	11·32 11·10
IVp	—	0·85	0·86 (S7)	$C_9H_{13}N_4O_4P.H_2O$ (290·2)	37·25 37·15	5·21 5·32	19·30 19·44	10·68 10·62
IVq	0·18	0·93	2·15 (S4)	$C_7H_9N_2O_6PLi_2$ (262·1)	— —	— —	10·69 10·46	11·84 11·59
IVr	0·29	0·92	3·05 (S4)	$C_8H_{11}N_2O_6PLi_2$ (276·1)	34·80 34·55	4·02 4·32	10·15 10·26	11·23 11·24
IVs	0·17	0·76	0·78 (S4)	$C_7H_{12}N_3O_5P.H_2O$ (267·3)[d]	31·46 31·70	5·28 5·07	15·72 16·05	11·61 11·41

TABLE II
(*Continued*)

Compound	R_F (S3)	E_{Up} (S4)	k (S)[a]	Formula (M.w.)	Calculated/Found			
					% C	% H	% N	% P
IVt	0·23	0·90	1·52 (S4)	$C_8H_{12}N_3O_5PNa_2 . 3\,H_2O$ (361·3)	—	—	11·63	8·59
					—	—	11·31	8·38
XIb	0·06	1·25 *	2·60 (S4)	$C_7H_9N_2O_6PLi_2$ (262·1)	—	—	10·69	11·84
					—	—	10·87	11·63

[a] $k = (t_r - t_o)/t_o$, where t_r is retention time, t_o is hold-up time; [b] calculated: 10·14% S; found: 10·06% S; [c] m.p. 214–215°C; [d] m.p. 226°C.

other biological studies. According to preliminary results, some of these compounds, particularly derivatives of adenine, 2,6-diaminopurine and guanine, show a significant antiviral activity against the whole spectrum of DNA viruses (HSV-1, HSV-2, VZV, CMV) and retroviruses (MSV, HIV) (refs[11–16]).

EXPERIMENTAL

Methods

Unless stated otherwise, the solutions were stripped of solvents at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography (TLC) on silica gel was carried out on Silufol UV 254 plates (Kavalier, Votice, Czechoslovakia) in systems S1 chloroform–methanol (9 : 1) and S2 chloroform–methanol (4 : 1). Paper chromatography was performed on a paper Whatman No 1 in system S3 (2-propanol–conc. aqueous ammonia–water 7 : 1 : 2). Paper electrophoresis was done on a Whatman No 3MM paper in a 0·1M buffer S4 (triethylammonium hydrogen carbonate) at 40 V cm^{-1}. HPLC analyses were carried out on columns (150 × 4 mm) of C18-silica gel Separon CGX (10 μ) in 0·05M buffer S4 containing 1% of acetonitrile (S5), 5% of acetonitrile (S6), 10% of acetonitrile (S7) and 5% of methanol (S8). Elution rate 1 ml min^{-1}, detection at 260 nm, Preparative chromatography on silica gel (30–40 μ) was performed either on columns or on loose layers (40 × 16 × 0·4 cm) of silica gel with an indicator (all products of Service Laboratories of this Institute). UV absorption spectra were measured on a PU 8 800 UV/VIS spectrophotometer (Pye–Unicam, Cambridge, Great Britain), NMR spectra were obtained with a Varian XL-2 000 instrument (chemical shifts δ in ppm, coupling constants J in Hz).

Starting Materials and Reagents

Dimethylformamide and acetonitrile (Janssen, Belgium) were dried over phosphorus pentoxide, distilled in vacuo and stored over molecular sieves. Bromotrimethylsilane, benzoic anhydride,

4-dimethylaminopyridine and adenine were Janssen products, uracil, thymine, cytosine, guanine, 4-aminopyrazolo[5,6*d*]pyrimidine and 2-aminopurine were purchased from Lachema (Czechoslovakia), 2-amino-6-chloropurine from Mack (F.R.G.), 6-methylthiopurine from Loba-Chemie (Austria) and 5-methylcytosine from Calbiochem (U.S.A.). 4-Methoxy-2-pyrimidone and 4-methoxy-5-methyl-2-pyrimidone were prepared according to ref.[17], 3-deazaadenine according to ref.[18] and N^2-acetylguanine according to ref.[19].

2,6-*Diaminopurine*. Dowex 50X8 (H^+-form) was gradually added to a stirred suspension of 2,6-diaminopurine hemisulfate hydrate (Sigma, U.S.A., 100 g, 0·46 mol) in water (300 ml) until dissolution. The suspension was applied on a column of the same ion-exchanging resin (400 ml) and the column was washed first with water until the UV absorption and conductivity of the eluate dropped to the original values, and then with 2·5% ammonia. The UV-absorbing ammonia eluate was taken down in vacuo, and the residue was codistilled with ethanol (4 × 100 ml) and pyridine (3 × 100 ml). After addition of anhydrous ether (300 ml), the product was filtered, washed with anhydrous ether (300 ml) and dried in vacuo (finally at 50°C/13 Pa over phosphorus pentoxide); yield 92%.

6-*Hydrazinopurine*. A mixture of 6-methylthiopurine (7·5 g, 45 mmol) and 85% hydrazine hydrate (20 ml) was heated in an autoclave to 120°C for 15 h. The reaction mixture was concentrated in vacuo, the residue was codistilled with water (4 × 50 ml), mixed with water (50 ml) and applied onto a column of Dowex 50X8 (H^+-form, 200 ml). The column was washed with water to loss of the UV absorption and the product was eluted with 2·5% aqueous ammonia. The UV-absorbing eluate was taken down, the residue was codistilled with ethanol (3 × 50 ml), mixed with ether, filtered and dried. Yield 3·75 g (55%) of slightly violet product (R_F 0·56, S1) which was used without further purification.

N^4-*Benzoylcytosine*. Cytosine (17·1 g, 0·154 mol) and 4-dimethylaminopyridine (2·5 g) were added to a solution of benzoic anhydride (38·4 g, 0·17 mmol) in acetonitrile (300 ml) and the reaction mixture was stirred and refluxed for 7 h. Ethanol (400 ml) was gradually added under stirring to the thick boiling reaction mixture. After standing at room temperature overnight, the solid was filtered, washed with ethanol and ether and dried; yield 30·1 g (91%) of chromatographically pure product.

N^4-*Benzoyl-5-methylcytosine*. A mixture of 4-methoxy-5-methyl-2-pyrimidone (4·0 g, 29 mmol) and 30% methanolic ammonia (70 ml) was heated in an autoclave to 120°C for 6 h. After cooling and evaporation, the residue was deionized on a column of Dowex 50X8 (H^+-form, 100 ml). The column was washed with water and the product was eluted with 2·5% aqueous ammonia. The UV-absorbing eluate was taken down and the residue crystallized from water on addition of ethanol (4 volumes), followed by ether (to turbidity). Yield 3·3 g (92%) of 5-methylcytosine, identical (TLC in S2) with a commercial sample (R_F 0·20). This product was mixed with benzoic anhydride (6·8 g, 30 mmol), 4-dimethylaminopyridine (0·5 g) and acetonitrile (80 ml) and refluxed with stirring for 7 h. Ethanol (50 ml) was added to the boiling mixture and, after cooling, the product was filtered, washed with ethanol and ether and dried. Yield 4·5 g (78%), R_F 0·32 (S2).

Diethyl 2-Chloroethoxymethylphosphonate (*IIb*)

Gaseous hydrogen chloride was introduced into a mixture of 2-chloroethanol (376 g, 4·66 mol) and 1,3,5-trioxane (140 g, 4·66 mol) for 10 h. The aqueous layer was separated and the remaining oil was dried over powdered calcium chloride under stirring and introduction of dry hydrogen. After 2 h the mixture was filtered and the filtrate destilled in vacuo; yield 432 g (74%) of 2-chloroethoxymethyl chloride, b.p. 50–55°C/2 kPa.

The thus-obtained compound (120 g, 1 mol) was placed into a flask equipped with a dropping funnel and a reflux condenser and triethyl phosphite (182·8 g, 1·1 mol) was added dropwise with stirring at 90°C at such a rate as to keep a rapid evolution of methyl chloride (90 min total). The mixture was then heated to 125°C for 4 h and distilled in vacuo, affording 209 g (90%) of compound *IIb*, b.p. 110°C/5 Pa. For $C_7H_{16}ClO_4P$ (236·5) calculated: 36·45% C, 6·99% H, 15·37% Cl, 13·43% P; found: 36·75% C, 7·70% H, 15·44% Cl, 13·43% P.

Dimethyl 2-Chloroethoxymethylphosphonate (*VIII*)

Trimethyl phosphite (33 ml, 0·28 mol) was added dropwise over 40 min under stirring to 2-chloroethoxymethyl chloride (35·6 g, 0·275 mol) at 90°C under reflux condenser. The mixture was heated to 120°C for additional 4 h and distilled in vacuo. Yield 38·4 g (69%) of compound *VIII*, b.p. 100°C/5 Pa. For $C_5H_{12}ClO_4P$ (202·5) calculated: 29·64% C, 5·97% H, 17·50% Cl, 15·29% P; found: 30·23% C, 6·91% H, 17·48% Cl, 15·52% P.

9-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (*IVa*)

Method A. Sodium hydride (2·4 g, 0·1 mol) was added to a suspension of 2,6-diaminopurine (15·0 g, 0·1 mol) in dimethylformamide (500 ml) and the mixture was stirred at 80°C for 1 h under exclusion of moisture. To the obtained solution of the sodium salt of the base was added a solution of compound *IIa* (39·4 g, 0·11 mol) in dimethylformamide (40 ml) and the stirred reaction mixture was heated to 100°C for 24 h. After evaporation of the solvent at 50°C/2 kPa, the residue was extracted with boiling chloroform (1 h total). The extract was taken down in vacuo and the residue was chromatographed on a column of silica gel (600 ml) in chloroform. The contaminants were removed by washing with chloroform and chloroform–methanol (49 : 1), elution with chloroform–methanol (19 : 1) afforded compound *IIIa*. The combined product fractions were taken down and the residue was crystallized from ethanol (with addition of the same volume of ether and then of light petroleum to turbidity) to give 16·0 g (47%) of compound *IIIa*. Its characteristics are given in Table I.

Bromotrimethylsilane (25 ml, 29 g, 190 mmol) was added to a suspension of compound *IIIa* (17·2 g, 50 mmol) in acetonitrile (100 ml) and the formed solution was set aside at room temperature for 2 days. The mixture was taken down in vacuo, the residue was codistilled with acetonitrile (2 × 50 ml) and mixed with 0·1M triethylammonium hydrogen carbonate pH 7·5. Triethylamine was gradually added until the mixture became homogeneous and constantly alkaline (pH 9). After 30 min, the solution was again taken down in vacuo, the residue was codistilled with methanol (2 × 100 ml) and dissolved in water (100 ml) on acidification with concentrated hydrochloric acid. This solution was applied on a column of Dowex 50X8(H^+-form, 300 ml) and the column was washed with water to drop of conductivity and UV-absorption of the eluate to the original values. The resin was then suspended in 5% aqueous ammonia (1 l), the suspension was stirred for 2 h, filtered and the resin washed with boiling water (2 l) which had been made alkaline with ammonia. The combined eluates were concentrated in vacuo and the residue was dissolved in water (100 ml) with addition of ammonia. The obtained solution was applied onto a column of Dowex 1X2 (acetate form, 200 ml). The column was washed with water to drop of UV absorption of the eluate to the original value and the Dowex was suspended in a mixture of water (500 ml) and formic acid (120 ml). The stirred mixture was taken to the boil, filtered and the resin was washed with 2M formic acid (2 l). The combined eluates were taken down in vacuo and the residue was codistilled with water (4 × 100 ml) to remove the residual formic acid. The residue was suspended in boiling water (200 ml), mixed with ethanol (1 200 ml) and ether was added to turbidity. After cooling, the crystalline product was filtered, washed with

ether and dried in vacuo, yield 14·4 g (100% based on *IIIa*) of compound *IVa* (for data see Table II). ^{13}C NMR spectrum (D_2O): 44·08 s (C-1'); 69·88 d (C—P, ^1J(C, P) = 128·2); 71·47 d (C-2', ^3J(C, P) = 11·9); 113·78 s (C-5); 141·66 s (C-8); 151·89 s (C-4); 156·95 s (C-6); 160·88 s (C-2).

Method B. Sodium hydride (9·6 g, 0·4 mol) was added to a stirred suspension of 2,6-diaminopurine (60 g, 0·4 mol) in dimethylformamide (2 l). The mixture was stirred at 80°C for 1 h (calcium chloride protective tube), then compound *IIb* (116 g, 0·5 mol) was added dropwise in the course of 30 min. The stirred mixture was heated to 80°C for 24 h (after 10 h another portion of compound *IIb* (23·2 g, 0·1 mol) was added). Further work-up of the mixture (evaporation, extraction with chloroform, chromatography on silica gel) was the same as described under *A*. The diester *IIIa* was obtained in a yield of 58% (90·5 g) and was identical with the product described above. Its conversion to compound *IVa* was executed in the same manner as described under *A*.

Method C. Sodium hydride (0·12 g, 5 mmol) was added to a suspension of 2,6-diaminopurine (0·75 g, 5 mmol) in dimethylformamide (30 ml) and the mixture was stirred at 80°C for 1 h under exclusion of moisture. A solution of compound *IIc* (1·50 g, 5·45 mmol) in dimethylformamide (10 ml) was added, the mixture was heated to 80°C for 16 h and then taken down at 40°C/13 Pa. The residue was extracted with boiling chloroform (300 ml), the extract was stripped of the solvent in vacuo and chromatographed on one plate of silica gel (S2). The zone of the product was eluted with methanol (300 ml) which was then evaporated and the residue was crystallized from ethanol–light petroleum; yield 0·80 g (47%) of compound *IIIa*, identical (TLC in S2) with the product prepared according to method *A*. The obtained product was mixed with acetonitrile (25 ml) and bromotrimethylsilane (1·5 ml) and allowed to stand for 2 days. After evaporation in vacuo, the residue was codistilled with acetonitrile (2 × 25 ml), taken up in 0·4M buffer S4 (50 ml) and set aside for 3 h. The solution was evaporated in vacuo, the residue was codistilled with methanol (2 × 50 ml) and deionized on a column of Dowex 50X8 (H^+-form, 100 ml). The ammonia eluate of the product was taken down and the residue was chromatographed on a column of Sephadex A-25 (HCO_3^--form, 150 ml), elution with a linear gradient 0·02 to 0·20M buffer S4 (à 1 l). The product-containing fractions (0·10—0·15 m buffer) were evaporated in vacuo, the residue was coevaporated with methanol (3 × 50 ml), dissolved in water (20 ml) and applied onto a column of Dowex 50X8 (Na^+-form, 100 ml). Elution with water and evaporation of the UV-absorbing eluate, followed by codistillation with ethanol and precipitation with ether (200 ml) from methanol (20 ml), afforded monohydrate of disodium salt of compound *IVa* (0·75 g, 92%, based on *IIIa*). The salt did not melt up to 300°C. For $C_8H_9N_6O_3Na_2.2\,H_2O$ (350·3) calculated: 24·00% N, 8·86% P; found: 24·20% N, 8·71% P. Its UV spectrum, chromatographic constants and E_{Up} values corresponded to those given for compound *IVa* in Table II.

7-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (*IVb*)
and 3-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (*IVc*)

The column of silica gel after elution of compound *IIIa* (see the preparation of compound *IVa* by method *A*) was washed with methanol (2 l), the eluate was evaporated in vacuo and the residue was allowed to stand with 1M sodium hydroxide (60 ml) at room temperature overnight. The mixture was acidified by addition of Dowex 50X8 (H^+-form) and the suspension was poured on a column of the same ion-exchanger (200 ml). After washing with water 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 dissolved in water (50 ml), was applied onto a column of Dowex 1X2 (acetate form, 500 ml). After washing with water to

drop of UV absorption to the original value, the material was eluted using a linear gradient of acetic acid (0—0·5M, à 2 l). The material, eluted with 0·10—0·12M acetic acid, after evaporation and crystallization from water afforded 1·3 g (4%) of compound *Vb*, the fraction obtained with 0·12—0·20M acid gave analogously 1·1 g (3·5%) of compound *Vc*, and the fractions from 0·20 to 0·35M acid afforded 0·6 g (2%) of compound *Va*.

N^9-*Derivative* Va: no melting up to 270°C; for $C_{10}H_{17}N_6O_4P$ (316·3) calculated: 37·96% C, 5·42% H, 26·57% N, 9·81% P; found: 38·14% C, 5·44% H, 26·64% N, 9·71% P. R_F 0·38 (S2), E_{Up} 0·42. UV-spectrum (pH 2): λ_{max} 254, 292 nm; λ_{min} 270 nm; (pH 7 and 12): λ_{max} 256, 282 nm; λ_{min} 265 nm (corresponds to compound *IIIa*).

N^7-*Derivative* Vb: m.p. 277°C. For $C_{10}H_{17}N_6O_4P$ (316·3) calculated: 37·96% C, 5·42% H, 26·57% N, 9·81% P; found: 38·19% C, 5·41% H, 26·32% N, 9·60% P. R_F 0·44 (S2), E_{Up} 0·30. UV-spectrum (pH 2): λ_{max} 224, 243 nm; λ_{min} 260 nm.

N^3-*Derivative* Vc: m.p. 194°C. For $C_{10}H_{17}N_6O_4P$ (316·3) calculated: 37·96% C, 5·42% H, 26·57% N, 9·81% P; found: 37·72% C, 5·43% H, 26·70% N, 9·88% P. R_F 0·38 (S2), E_{Up} 0·47. UV-spectrum (pH 2): λ_{max} 245, 283 nm, λ_{min} 260 nm; (pH 7 and 12): λ_{max} 293 nm, λ_{min} 262 nm.

Bromotrimethylsilane (4 ml) was added to a suspension of compound *Vb* or *Vc* (0·95 g, 3 mmol) in acetonitrile (40 ml) and the solution was set aside at room temperature for 48 h. After evaporation in vacuo, the residue was codistilled with acetonitrile (2 × 20 ml), mixed with 0·4M buffer S4 (100 ml), adjusted to pH 9 with triethylamine and allowed to stand for 30 min. The mixture was evaporated in vacuo, the residue was codistilled with methanol (2 × 50 ml), dissolved in water (20 ml), applied to a column of Dowex 50X8 (Na^+-form, 200 ml) and eluted with water. The UV-absorbing eluate was concentrated in vacuo and the residue was codistilled with ethanol and precipitated with ether (200 ml) from ethanol (20 ml). The obtained product was collected on filter, washed with ether and dried in vacuo; yield 0·77 g (76%) of dihydrate of disodium salt of compound *IVb* or 0·86 g (85%) of dihydrate of disodium salt of compound *IVc*. Characteristic data of these products are given in Table II.

9-(2-Phosphonylmethoxyethyl)hypoxanthine (*IVd*)

To a solution of compound *I* (5 mmol) in 80% acetic acid (60 ml) was added 3-methylbutyl nitrite (5 ml) and the mixture was stirred in a stoppered flask for 72 h at room temperature. After evaporation in vacuo, the residue was codistilled with water (5 × 50 ml), dissolved in water (50 ml) and applied onto a column of Dowex 50X8 (H^+-form, 200 ml). The column was first washed with water to drop of UV absorption and conductivity to the original values, and then with water–methanol (9 : 1). The UV-absorbing eluate was concentrated in vacuo, the residue was codistilled with ethanol (3 × 50 ml) and crystallized from methanol (ether added to turbidity). Yield 1·2 g (93%) of compound *IVd* (for characteristic data see Table II).

9-(2-Phosphonylmethoxyethyl)-2-aminopurine (*IVe*)

The reaction was carried out with 2-aminopurine (0·67 g, 5 mmol) as described in the preparation of compound *IVa* (method *C*) and afforded 0·67 g (40%) of compound *IIIe* as an evaporation residue which was further converted into compound *IVe* using the above-cited procedure. Deionization, chromatography on Sephadex A-25 and conversion into the sodium salt afforded 0·52 g (77%, based on compound *IIIe*) of monohydrate of disodium salt of compound *IVe* whose characteristic data are given in Table II.

9-(2-Phosphonylmethoxyethyl)-6-methylthiopurine (*IVf*)

A mixture of 6-methylthiopurine (6·65 g, 40 mmol), sodium hydride (0·96 g, 40 mmol) and dimethylformamide (200 ml) was stirred at 50°C for 1 h under exclusion of moisture. Compound *IIb* (11·6 g, 50 mmol) was added and the stirred mixture was heated to 50°C for 22 h. Further work-up procedure was the same as described for compound *IVa* (method *B*). Chromatography on silica gel gave 10·2 g (71%) of compound *IIIf* as a colourless oil. This product was dissolved in acetonitrile (150 ml), mixed with bromotrimethylsilane (10 ml, 11·6 g, 76 mmol) and set aside overnight. The reaction mixture was processed as described for compound *IVa*. The crude triethylammonium salt of compound *IVf* was dissolved in water (50 ml), applied onto a column of Dowex 50X8 (Li^+-form, 150 ml) and eluted with water. The UV-absorbing eluate was concentrated in vacuo almost to dryness and the residue was codistilled with ethanol (3 × 100 ml). The material was stirred with ethanol (100 ml) for 15 min and mixed with the same volume of acetone. The product was filtered, washed successively with ethanol–acetone (1 : 1), acetone and ether and dried in vacuo. Yield 6·9 g (77% based on compound *IIIf*) of dilithium salt of compound *IVf*, homogeneous according to paper chromatography and HPLC (Table II). Its UV spectrum corresponded to a 9-alkyl-6-methylthiopurine. ^{1}H NMR (D_2O): 2·55 s, 3 H (S—CH_3); 3·68 d, 2 H (9-CH_2, J(P, CH) = 8·7); 4·00 t, 2 H (2′-CH_2, J = 5·0); 8·35 s, 2 H (2-H + 8-H).

9-(2-Phosphonylmethoxyethyl)-6-hydrazinopurine (*IVg*)

The reaction was carried out with sodium salt of 6-hydrazinopurine (8 mmol) and compound *IIc* (2·8 g, 10 mmol) in dimethylformamide (80 ml) at 100°C for 12 h as described for compound *IVa* (method C). The compound *IIIg* was isolated by chromatography on a plate of silica gel in system S2; yield 9·80 g (2·4 mmol, 30%). Further work-up according to the above-cited procedure (deionization, chromatography on Sephadex A-25) afforded disodium salt of compound *IVg* (88% based on compound *IIIg*) which is characterized in Table II.

9-(2-Phosphonylmethoxyethyl)-6-methylpurine (*IVh*)

The title compound was prepared from sodium salt of 6-methylpurine (2 mmol) and compound *IIc* (2·1 mmol) in dimethylformamide (20 ml) as described for compound *IVa* (method *C*). Chromatography on a plate of silica gel in the system S2 afforded 0·30 g (46%) of compound *IIIh* which was converted into triethylammonium salt of compound *IVh* by the above-described procedure (without deionization on Dowex 50). The triethylammonium salt, obtained by evaporation of fractions from chromatography on Sephadex A-25 (fractions eluted with 0·08—0·10 mol . l^{-1} buffer S4) was dissolved in water (10 ml), applied onto a column of silica gel C-18 (30 μ, 200 ml) and eluted with water. After washing out the salts, the compound *IVh* was eluted (with retention). The product fractions were taken down and the residue was converted into the sodium salt (column of Dowex 50X8 (Na^+-form, 50 ml)) as described above. Yield 0·20 g (63%) of dihydrate of disodium salt of compound *IVh*, homogeneous according to paper chromatography and HPLC (see Table II).

7-(2-Diethoxyphosphonylmethoxyethyl)- (*IIIi*) and
9-(2-Diethoxyphosphonylmethoxyethyl)-2-amino-6-chloropurine (*IIIj*)

A mixture of 2-amino-6-chloropurine (6·0 g, 35 mmol), anhydrous potassium carbonate (9·8 g, 70 mmol), dimethylformamide (140 ml) and compound *IIb* (11·4 g, 50 mmol) was stirred under exclusion of moisture (calcium chloride tube) at 80°C for 16 h. The hot mixture was filtered, the solid was washed with dimethylformamide (50 ml) and the filtrate was evaporated at 40°C/

13 Pa. The residue was dissolved in chloroform, applied onto a column of silica gel (400 ml) and both the reaction products were obtained by elution with chloroform–methanol (49 : 1). Evaporation of the corresponding fractions and crystallization from ether–light petroleum afforded 8·1 g (64%) of the 9-isomer *IIIj* and 1·5 g (12%) of the 7-isomer *IIIi*. (For data see Table I).

N^7-*Derivative* IIIi: ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·14 s, 6 H (CH_2CH_3 J = 7·0); 3·83 d, 2 H (P—CH_2, J(P—CH) = 8·3); 3·87 t, 2 H (2′-CH_2, J = 5·0); 3·90 dq, 4 H (CH_2—CH_3. J(CH_2CH_3) = 7·0, J(P—OCH) = 8·2; 4·49 t, 2 H (N—CH_2, J = 5·0); 6·61 br s, 2 H (NH_2); 8·29 s, 1 H (8-H). UV-spectrum (methanol): λ_{max} 324 nm.

N^9-*Derivative* IIIj: ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·13 s, 6 H (CH_2CH_3, J = 7·0); 3·83 d, 2 H (P—CH_2, J(P—CH) = 8·1); 3·88 t, 2 H (2′-CH_2, J = 5·0); 3·92 dq, 4 H (CH_2—CH_3, J = 7·0, J(P—OCH) = 7·9); 4·24 t, 2 H (N—CH_2, J = 5·0); 6·90 br s, 2 H (NH_2); 8·06 s, 1 H (8-H). UV-spectrum (methanol): λ_{max} 310 nm.

9-(2-Diethoxyphosphonylmethoxyethyl)-N^2-acetylguanine (*IIIm*)

Sodium hydride (0·48 g, 20 mmol) was added with ice-cooling to a suspension of N^2-acetylguanine (3·9 g, 20 mool) in dimethyl formamide (100 ml). After stirring under exclusion of moisture at 0°C for 1 h, compound *IIc* (5·6 g, 20 mmol) was added, the mixture was stirred at room temperature for 2 h and at 70°C for 12 h and then taken down at 50°C/13 Pa. The crude product was extracted from the residue with boiling chloroform (500 ml total) and purified by chromatography on a column of silica gel (200 ml) in chloroform–methanol (19 : 1). Crystallization from ethyl acetate–ether afforded 1·5 g (20%) of compound *IIIm* (see Table I); UV spectrum (methanol): λ_{max} 268 nm (ε_{max} 15 500).

9-(2-Phosphonylmethoxyethyl)guanine Ethyl Ester (*VI*)

A mixture of compound *IIIj* (7·4 g, 20 mmol), dioxane (120 ml) and 1M sodium hydroxide (120 ml) was stirred to homogeneity and then set aside overnight. The solution was neutralized by addition of Dowex 50X8 (H^+-form), made alkaline with triethylamine, filtered, the solid was washed with water (100 ml) and the combined filtrates were evaporated in vacuo. The dry residue was refluxed with 1M hydrochloric acid (250 ml) for 2 h and the mixture was neutralized with ammonia and taken down in vacuo. The residue, dissolved in water (100 ml), was applied onto a column of Dowex 50X8 (H^+-form, 250 ml) and eluted with water. The product was eluted with considerable retention and was obtained by evaporation of the UV-absorbing fractions, codistillation with ethanol and crystallization from ethanol–ether. Yield 5·2 g (82%) of compound *VI*, m.p. 195°C, R_F 0·42 (S1), E_{Up} 0·52. For $C_{10}H_{16}N_5O_5P$ (317·3) calculated: 37·85% C, 5·08% H, 22·08% N, 9·78% P; found: 37·55% C, 5·01% H, 21·82% N, 9·86% P. UV-spectrum (pH 2): λ_{max} 254 nm, λ_{infl} 265 nm. HPLC: k = 4·3 (S6). ^{1}H NMR spectrum (D_2O + NaOD): 1·05 t + 3·71 br pent, 5 H (C_2H_5, J(CH_2CH_3) = 7·0, J(P—OCH) = 8·0, J = 7·0); 3·67 d, 2 H (P—CH_2, J(P—CH) = 8·0); 3·91 t, 2 H (2′-CH_2, J = 4·6); 4·23 t, 2 H (N—CH_2, J = 4·6); 7·75 s, 1 H (8-H).

7-(2-Phosphonylmethoxyethyl)guanine (*IVk*)

A solution of compound *IIIi* (1·1 g, 3 mmol) in a mixture of dioxane (20 ml) and 1M sodium hydroxide (20 ml) was allowed to stand overnight, neutralized with Dowex 50X8 (H^+-form), made alkaline with triethylamine, filtered and the solid was washed with water (100 ml). The filtrate was taken down in vacuo, the residue was heated with 1M hydrochloric acid (100 ml) to 100°C for 2 h, neutralized with ammonia and evaporated in vacuo. The residue was deionized

on a column of Dowex 50X8 (H^+-form, 200 ml) and the ammonia eluate was taken down. The residue (compound *Vk*) was codistilled with ethanol (2 × 50 ml), dried over phosphorus pentoxide at 13 Pa, mixed with bromotrimethylsilane (3 ml, 22·7 mmol) and set aside overnight. After evaporation in vacuo, the material was further worked up as described for compound *IVa* (method *C*). The sodium salt of compound *IVk* (dihydrate) was obtained by crystallization from methanol–acetone (ether added to turbidity); yield 0·95 g (86%). The compound is characterized in Table II.

9-(2-Phosphonylmethoxyethyl)guanine (*IVl*)

Method *A*. A mixture of compound *VI* (3·2 g, 10 mmol), acetonitrile (200 ml) and bromotrimethylsilane (20 ml) was set aside at room temperature for 48 h. After evaporation in vacuo, the residue was codistilled with acetonitrile (2 × 50 ml), mixed with water (150 ml) and treated with triethylamine to alkaline reaction. After 2 hours the mixture was taken down in vacuo and deionized on a column of Dowex 50X8 (H^+-form, 400 ml). The ammonia eluate was evaporated and the residue was dissolved in water (50 ml) and applied onto a column of Dowex 50X8 (Na^+-form, 200 ml). Elution with water, evaporation of the UV-absorbing eluate, codistillation of the residue with ethanol, precipitation from methanol (40 ml) with ether (200 ml) and drying in vacuo afforded chromatographically pure monohydrate of disodium salt of compound *IVl*; yield 3·4 g (97%) (see Table II).

Method *B*. Compound *IIIm* (3 mmol) was treated with bromotrimethylsilane as described for the preparation of compound *IVa* (method *C*). After treatment of the evaporation residue with a solution of the buffer S4 and evaporation, the residue was allowed to stand with 20% aqueous ammonia for 48 h, evaporated and deionized on a column of Dowex 50X8 (H^+-form). The ammonia eluate was taken down, chromatographed on Sephadex A-25, and converted into the sodium salt (vide supra). Yield 1·0 g (90%) of dihydrate of disodium salt *IVl* (see Table II).

7-(2-Phosphonylmethoxyethyl)-4-aminopyrazolo[5,6*d*]pyrimidine (*IVn*)

A solution of compound *IIc* (8·5 g, 31 mmol) in dimethylformamide (25 ml) was added dropwise during 2 h at 80°C to a stirred suspension of sodium salt of 4-aminopyrazolo[5,6*d*]pyrimidine (25 mmol) in dimethylformamide (200 ml), prepared as described for compound *IVa*. After stirring for 5 h at 80°C (calcium chloride tube), the mixture was worked up as described for compound *IIIa* (method *A*). Chromatography on silica gel and crystallization from ether afforded compound *IIIn* (3·9 g, 47%) (for characteristic data see Table II).

A mixture of compound *IIIn* (3·3 g, 10 mmol), acetonitrile (20 ml) and bromotrimethylsilane (15 ml) was set aside overnight and processed in the same manner as described for compound *IVl* (method *A*). Yield 3·0 g (95%) of disodium salt of compound *IVn*, homogeneous according to paper chromatography, HPLC and electrophoresis (see Table II).

7-(2-Phosphonylmethoxyethyl)-4-hydroxypyrazolo[5,6*d*]pyrimidine (*IVo*)

A solution of sodium salt of compound *IVn* (0·95 g, 3 mmol) and 3-methylbutyl nitrite (4 ml) in 80% acetic acid (50 ml) was set aside at room temperature in a stoppered flask for 60 h and then processed in the same manner as described for compound *IVd* (a column of Dowex 50X8 (H^+-form, 100 ml, elution with water). After evaporation in vacuo, the eluted product was codistilled with ethanol (2 × 50 ml), mixed with ethanol–ether and collected on filter; yield 0·50 g (61%) of compound *IVo* (free acid), homogeneous according to paper chromatography, HPLC and electrophoresis (see Table II).

9-(2-Phosphonylmethoxyethyl)-3-deazaadenine Ethyl Ester (*Vp*)

Compound *IIa* (3·3 g, 9 mmol) was added at 80°C to a suspension of sodium salt of 3-deazaadenine (7·5 mmol) in dimethylformamide (30 ml), prepared as described in the preparation of compound *IVa*. The mixture was stirred at 100°C for 6 h under exclusion of moisture and taken down at 50°C/2 kPa. The residue was codistilled with toluene (2 × 50 ml), extracted with boiling chloroform (250 ml total), the chloroform was evaporated and the residue was chromatographed on a column of silica gel (100 g) in chloroform. The product *IIIp* was eluted with chloroform–ethanol (19 : 1) and the solvents were evaporated. The residue was warmed to 40°C with 0·2M sodium hydroxide (40 ml) for 12 h, the mixture was acidified by addition of Dowex 50X8 (H^+-form), the suspension was layered onto a column of the same ion-exchanger (100 ml) and the column was washed with water to drop of UV absorption and conductivity of the eluate to the original values. Elution with 2·5% ammonia gave UV-absorbing fraction which was taken down in vacuo and the residue was chromatographed on Dowex 1X2 (acetate form, 100 ml) with a linear gradient of acetic acid (0—0·5M, 1 l each). The product fraction was evaporated in vacuo, the residue was codistilled with water (3 × 50 ml) and ethanol and crystallized from ethanol–ether; yield 0·56 g (25%) of compound *Vp*, m.p. 220—223°C. For $C_{11}H_{17}N_4O_4P.2\,H_2O$ (336·2) calculated: 39·29% C, 6·30% H, 16·65% N, 9·22% P; found: 39·69% C, 6·20% H, 16·71% N, 9·05% P. ^{1}H NMR spectrum (D_2O): 0·88 t, 3 H (C—CH_3), J = 7·2); 3·46 d, 2 H (P—CH_2, J(P—CH) = 8·6); 3·55 br pent, 2 H (CH_2CH_3, J(P—OCH) = 8·0, J(CH_2CH_3) = 7·2); 3·77 t, 2 H (2·-CH_2, J = 5·0); 4·31 t, 2 H (N—CH_2, J = 5·0); 7·02 d, 1 H (3-H, J(2, 3) = 7·3); 7·40 d, 1 H (2-H, J(2, 3) = 7·3); 8·27 s, 1 H (8-H). UV spectrum (pH 7): λ_{max} 261·5 nm (ε_{max} 8 500); (pH 13): λ_{max} 265·5 nm (ε_{max} 8 700). E_{Up} 0·51; HPLC: k = 2·76 (S6).

9-(2-Phosphonylmethoxyethyl)-3-deazaadenine (*IVp*)

Bromotrimethylsilane (1 ml) was added to a suspension of compound *Vp* (0·45 g, 1·5 mmol) in acetonitrile (10 ml) and the mixture was stirred in a stoppered flask for 18 h. The further work-up was carried out as described for compound *IVa* (method *C*). After hydrolysis, the crude product was purified by chromatography on Dowex 1X2 (acetate form, 100 ml); after washing with water to drop of the UV absorption to the original values, the product was obtained by elution with 0·5M acetic acid. The UV-absorbing eluate was evaporated in vacuo, the residue was codistilled with water (3 × 20 ml) and crystallized from water (with addition of 4 volumes of ethanol and then ether to turbidity) to give 0·30 g (71%, based on compound *Vp*) of compound *IVp*, homogeneous according to HPLC and electrophoresis (see Table II).

1-(2-Diethoxyphosphonylmethoxyethyl)uracil (*IIIq*) and
1,3-Bis(2-diethoxyphosphonylmethoxyethyl)uracil (*XIa*)

Compound *IIa* (14·9 g, 40 mmol) in dimethylformamide (30 ml) was added at 80°C to a suspension of sodium salt of uracil (40 mmol) in dimethylformamide (200 ml), prepared from uracil using the procedure described for compound *IVa*. The mixture was stirred at 100°C for 18 h under exclusion of moisture and processed as described for compound *IVa* (method *A*). Chromatography on silica gel (300 ml) in chloroform and then chloroform–methanol (49 : 1) afforded compound *XIa* (3·75 g, 19%) as a colourless oil and then compound *IIIq* (5·40 g, 44%, colourless oil). For their characteristics see Table I.

Compound IIIq. ^{1}H NMR spectrum ($CDCl_3$): 1·33 t, 6 H (CH_2CH_3, J(CH_2CH_3) = 7·0); 3·80 d, 2 H (P—CH_2, J(P—CH) = 8·5); 4·15 dq, 4 H (CH_2CH_3, J(CH_2CH_3) = 7·0, J(P—OCH) = (8·2); 3·75—4·0 m, 4 H (N—CH_2 + O—CH_2); 5·65 br d, 1 H (5-H, J(5, NH) = 1·0, J(5, 6) =

= 8·0); 7·35 d, 1 H (6-H, $J(6, 5) = 8·3$); 9·94 br s, 1 H (NH). UV-spectrum (pH 2, 7): λ_{max} 265 nm, (pH 12): λ_{max} 265 nm (25·5% hypochromism).

Compound XIa: ^{1}H NMR spectrum ($CDCl_3$): 1·33 t, 12 H (CH_2CH_2, $J = 7·0$); 3·79 d + + 3·86 d, 2 × 2 H (P—CH_2, J(P—CH) = 8·5); 4·14 dq + 4·15 dq, 4 × 2 H (CH_2—CH_3, J(P—OCH) = 8·2); 3·75—4·0 m, 8 H (N—CH_2 + O—CH_2); 5·67 d, 1 H (5-H, $J(5, 6) = 8·0$); 7·29 d, 1 H (6-H). UV-spectrum (pH 2, 7, and 12): λ_{max} 267 nm (no hypochromism at pH 12).

1-(2-Phosphonylmethoxyethyl)uracil (*IVq*)

A mixture of compound *IIIq* (5·4 g, 17·5 mmol), acetonitrile (100 ml) and bromotrimethylsilane (5 ml, 38 mmol) was set aside overnight and the reaction mixture was then worked up as described for compound *IVf*. Yield 3·4 g (75%) of dilithium salt of compound *IVq*, homogeneous according paper chromatography, HPLC and electrophoresis (Table II).

1,3-Bis(2-phosphonylmethoxyethyl)uracil (*XIb*)

Bromotrimethylsilane (4 ml, 30 mmol) was added to a solution of compound *XIa* (3·5 g, 7 mmol) in acetonitrile (12 ml), the mixture was allowed to stand overnight and processed as described for compound *IVf*. Yield 2·9 g of lithium salt of compound *XIb* (Table II).

1-(2-Phosphonylmethoxyethyl)thymine (*IVr*)

Compound *IIb* (18·2 g, 78·5 mmol) was added at 80°C to a solution of sodium salt of 4-methoxy--5-methyl-2-pyrimidone (50 mmol) in dimethylformamide (300 ml) and the mixture was heated to 100°C for 15 h. The solvent was evaporated at 50°C/2 kPa, the residue was codistilled with toluene (2 × 200 ml) and taken up in boiling chloroform (1 l). After evaporation of the chloroform, the residue was chromatographed on a column of silica gel (500 ml) in chloroform. The principal reaction product of R_F 0·43 (S1) (4-O-methyl derivative of compound *IIIr*) was obtained as a colourless oil (7·6 g, 45%). UV spectrum (methanol): λ_{max} 286 nm.

This product was dissolved in acetonitrile (200 ml) and allowed to stand with bromotrimethylsilane (10 ml) at room temperature overnight. After evaporation in vacuo, the residue was dissolved in water (80 ml), made alkaline with triethylamine and the solution was again taken down. The residue was refluxed with 80% acetic acid (150 ml) for 7 h, the mixture was evaporated and the residue codistilled with water (5 × 50 ml). The product was converted into the dilithium salt of compound *IVr* (5·9 g, 95%) as described for compound *IVf* (Table II).

1-(2-Phosphonylmethoxyethyl)cytosine (*IVs*)

Sodium hydride (0·96 g, 40 mmol) was added to a suspension of N^4-benzoylcytosine (8·6 g; 40 mmol) in dimethylformamide (150 ml) and the mixture was stirred at room temperature for 1 h under exclusion of moisture. The formed solution was mixed with compound *IIa* (16·5 g, 45 mmol) in dimethylformamide (20 ml), the mixture was heated to 100°C for 10 h and then taken down at 50°C/13 Pa. The residue was taken up in boiling chloroform (500 ml), the chloroform was evaporated and the residue was allowed to stand overnight with 0·1M methanolic sodium methoxide (200 ml). After neutralization with Dowex 50X8 (H^+-form) and alkalization with triethylamine, the mixture was filtered, the solid was washed with 1% methanolic triethylamine (300 ml) and the filtrate was taken down in vacuo. The residue was dried by codistillation with toluene (3 × 100 ml) and suspended in acetonitrile (200 ml). After addition of bromotrimethylsilane (15 ml, 114 mmol), the mixture was stirred to homogeneity and left to stand overnight.

The solvent was evaporated, the residue was codistilled with acetonitrile (50 ml) and dissolved in water (80 ml). The acidic solution was neutralized with ammonia, concentrated in vacuo to half of the original volume and applied onto a column of Dowex 50X8 (H^+-form, 200 ml). After washing with water (1 l) and 20% aqueous methanol to drop of UV absorption to the original value, the product was eluted with 2·5% ammonia. The UV-absorbing fraction was evaporated in vacuo and the residue was chromatographed on a column of Dowex 1X2 (acetate form, 200 ml), first in water to remove the salts and then in a linear gradient (0—0·2 mol l^{-1}) of acetic acid (à 2 l). The product was eluted with 0·09—0·16 mol l^{-1} acetic acid. After evaporation and codistillation with water (3 × 50 ml), the residue was dissolved in boiling water and mixed with fourfold volume of ethanol and one volume of ether. The product crystallized at 0°C overnight, yield 5·5 g (51%) of monohydrate of compound *IVs*, homogeneous according to the usual criteria (Table II).

1-(2-Phosphonylmethoxyethyl)-5-methylcytosine (*IVt*)

A solution of sodium salt of N^4-benzoylcytosine (9 mmol) in dimethylformamide (60 ml) was prepared in the same manner as described for the preparation of compound *IVs*. After addition of compound *IIc* (3 g, 11 mmol) the mixture was stirred at 80°C for 14 h and the solvent was evaporated at 40°C/13 Pa. The residue was extracted with boiling chloroform (300 ml), the solvent was evaporated and the remaining material was purified by chromatography on a column of silica gel (100 ml) in chloroform, affording 2·15 g (56%) of N^4-benzoyl derivative of compound *IIIt* (R_F 0·80, S1) as a colourless oil. This product was mixed with acetonitrile (50 ml) and bromotrimethylsilane (2 ml, 15 mmol) and set aside for 24 h. The mixture was taken down in vacuo, the residue was codistilled with acetonitrile (3 × 20 ml) and mixed with 0·4M buffer S4 (25 ml). The mixture was again evaporated in vacuo, the residue was codistilled with ethanol and briefly boiled with 0·1M methanolic sodium methoxide. After standing for 24 h, the mixture was neutralized with Dowex 50X8 (H^+-form), made alkaline with triethylamine, filtered, the solid washed with methanol (200 ml) and the filtrate taken down in vacuo. The residue was partitioned between water (50 ml) and ether (3 × 25 ml) and the aqueous phase was deionized on a column of Dowex 50X8 (H^+-form, 100 ml). The ammonia eluate was evaporated and converted into the sodium salt as described for compound *IVa* (method *C*). Yield 1·0 g (56%, based on N^4-benzoyl derivative of *IIIt*) of trihydrate of disodium salt of compound *IVt* (see Table II).

Reaction of Sodium Salt of Adenine with Dimethyl 2-Chloroethoxymethylphosphonate (*VIII*)

Compound *VIII* (34·4 g, 0·17 mol) was added at 80°C to a suspension of sodium salt of adenine (0·15 mol) in dimethylformamide (800 ml), prepared as described in the preparation of compound *IVa*. The mixture was heated to 100°C for 6 h under exclusion of moisture. After cooling, the mixture was filtered from the insoluble material, the filtrate was taken down at 50°C/2 kPa and the residue was crystallized from methanol. The product was filtered, washed with methanol and recrystallized from water, affording 6·7 g (30%) of 9-methyladenine (*X*), m.p. 275°C, identical (HPLC, k 1·74 in S7 and TLC, R_F 0·40 in S2) with an authetic sample. UV spectrum (pH 2, 7 and 12): λ_{max} 263 nm. ^{13}C NMR spectrum (hexadeuteriodimethyl sulfoxide): 29·74 ($N—CH_3$); 120·3 (C-5); 141·89 (C-8); 150·23 (C-5); 141·89 (C-8); 150·23 (C-4); 152·70 (C-2); 156·13 (C-6); ^{1}H NMR spectrum: 3·71 s, 3 H ($N—CH_3$); 7·17 br s, 2 H (NH_2); 8·06 + 8·13, 2 s, 2 H (2-H + 8-H).

The methanolic mother liquor from crystallization of compound *X* was evaporated in vacuo and the residue was chromatographed on a column of silica gel (300 ml) in chloroform. Elution

with chloroform–methanol (49 : 1), followed by crystallization from ethyl acetate–ether, afforded 4·9 g (11%) of dimethyl ester *IXa*, m.p. 254–255°C. For $C_{10}H_{16}N_5O_4P$ (301·3) calculated: 39·86% C, 5·35% H, 23·25% N, 10·30% P; found: 40·04% C, 5·27% H, 23·03% N, 10·11% P, R_F 0·50 (S1). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·58 d, 6 H (P—OCH_3; J(P—OCH) = 10·5); 3·88 t, 2 H (2′-CH_2, J = 5·1); 3·90 d, 6 H (P—CH_2, J(P—CH) = 8·4) 4·33 t, 2 H (N—CH_2, J = 5·1); 7·19 br s, 2 H (NH_2); 8·03 s + 8·14 s, 2 H (2-H + 8-H).

The chromatography was continued using methanol (2 l) as eluant. The solvent was evaporated and the residue was allowed to stand overnight with 0·5M sodium hydroxide (100 ml). The mixture was neutralized with Dowex 50X8 (H^+-form) and applied onto a column of the same ion-exchanging resin (200 ml). After washing out the salts and neutral UV-absorbing material with water, the compound *IXb* was eluted (considerable retention). Evaporation of water, codistillation of the residue with ethanol and crystallization from methanol–ether afforded 3·5 g (8%) of compound *IXb*, m.p. 204–205°C. For $C_9H_{14}N_5O_4P.H_2O$ (305·3) calculated: 35·40% C, 5·28% H, 22·95% N, 10·11% P; found: 35·71% C, 5·40% H, 22·87% N, 10·11% P. UV spectrum (pH 2): λ_{max} 263 nm (independent of pH). HPLC: k = 5·02 (S6).

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