SYNTHESIS OF (3-HYDROXY-2-PHOSPHONYLMETHOXYPROPYL) DERIVATIVES OF HETEROCYCLIC BASES*

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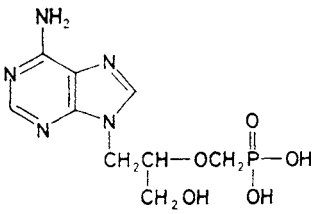
Analogs of the antiviral 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA, *I*), containing modified heterocyclic base, were prepared from racemic or (*S*)-N-(2,3-dihydroxypropyl) derivatives *II*. Compounds *II* are heated with chloromethylphosphonyl dichloride (*XVII*), the formed chloromethylphosphonylester chlorides of compounds *II* react with water to give a mixture of 2′- and 3′-chloromethylphosphonyl derivatives *XVIII* and *XIX*, respectively, which on isomerization by boiling with water in the arising acidic medium affords predominantly the 3′-isomer *XIX*. Treatment of this isomeric mixture with aqueous sodium hydroxide yields a mixture of 2′-O-phosphonylmethyl ethers (predominating, *XXI*) and 3′-O-phosphonylmethyl ethers of compounds *II* (*XX*). This approach has been applied to the synthesis of isomeric mixtures in the racemic as well as in the (*S*)-series derived from C-2, C-8 and N-6 substituted derivatives of adenine, from hypoxanthine and additional 6-substituted derivatives of purine, from guanine, 3-deazaadenine and other modified purine bases, from uracil, cytosine, their 5-methyl derivatives and 5-fluorouracil. Regioselective synthesis of compounds *XXI* was performed for biologically active derivatives (derivative of 2-aminoadenine (*XXIe*), guanine (*XXIn*), 3-deazaadenine (*XXIp*) and cytosine (*XXIt*)) as well as some other compounds (derivative of hypoxanthine (*XXIj*), uracil (*XXIr*), thymine (*XXIs*) and 5-methylcytosine (*XXIu*)): the former were obtained either from 3′-O-chloromethylphosphonyl derivatives *XIX*, isolated from the above-mentioned mixtures by ion-exchanger chromatography or HPLC, or by regioselective substitution, whereas the latter compounds were prepared by deamination (compound *XXIj* from adenine derivative *I* or the uracil and thymine derivatives *XXIr* and *XXIu* from the cytosine derivatives *XXIt* and *XXIu*). N-(*S*)-(3-Hydroxy-2-benzoyloxypropyl) derivative of N^4-benzoylcytosine (*XXIX*) and N^2-benzoylguanine (*XXIV*), obtained from compounds *IIn* and *IIt* by successive N-benzoylation, reaction with dimethoxytrityl chloride, benzoylation and mild acid treatment, were subjected to reaction with the chloride *XVII* and subsequent neutral and alkaline hydrolysis (compound *XXIV*), or to reaction with sodium methoxide followed by treatment with bromotrimethylsilane (compound *XXIX*), being thus converted into 1-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine (*XXIt*, HPMPC) and 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)guanine (*XXIn*, HPMPG), respectively. The starting compounds (*S*)-*II* were synthesized from sodium salt of the corresponding heterocyclic base by reaction with 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-(*R*)-glycerol (*IIIa*) (the (*RS*)-derivatives by reaction with 4-chloromethyl-2,2-dimethyl-1,3-dioxolane (*IIIb*)), followed by acid hydrolysis.

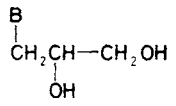
* Part VII of the series Acyclic Nucleotide Analogues; Part VI: Collect. Czech. Chem. Commun. *54*, 2190 (1989).

In one of our previous communications of this series[1] we described syntheses of a new acyclic nucleotide analog exhibiting an unusually interesting antiviral activity against DNA-viruses[2-5], 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA, *I*). Studies on structural modifications in the side-chain in this compound[6] have proven a very narrow margin of modification in which the biological effect remains preserved: of all structural modification types studied, only 9-(2-phosphonylmethoxyethyl)adenine (PMEA)[7] showed effects analogous to those of the parent compound[2,3]. Therefore, our further investigation of structure–activity relationships concerning this group of nucleotide antimetabolites, was necessarily directed to the effect of another structural parameter in the molecule of compound *I*, i.e., structural modification of the heterocyclic base. In the series of phosphonylmethyl ethers derived from N-(2,3-dihydroxypropyl) derivatives of heterocyclic bases, the present work concerns exclusively N-(3-hydroxy-2-phosphonylmethoxypropyl) isomers because both the enantiomers of the isomeric 9-(2-hydroxy-3-phosphonylmethoxypropyl)adenine did not exhibit any biological effect[2]. Although it has been established that only the (*S*)-enantiomer of compound *I* is responsible for the biological activity[2], its racemate is active, too; therefore we restricted this study to a broader selection of better accessible modified racemic compounds. Only in cases where the racemate demonstrated biological activity, the pure (*S*)-enantiomers were also synthesized.

Compounds of the type *I* can be prepared by two synthetic pathways: one of them, which has been methodically studied in detail for the synthesis of compound *I* (ref.[1]), starts from the easily accessible (*RS*)- or (*S*)-N-(2,3-dihydroxypropyl) derivatives of heterocyclic bases (vide infra); the phosphonylmethyl ether group is subsequently attached to the hydroxyl functionality in the side-chain. The second pathway makes use of a preformed organophosphorus synthon, capable of alkylating the heterocyclic base[8,9]. Although the latter method might seem to be more suitable for the purpose, it was abandoned because such synthon, particularly that required for the synthesis of (*S*)-enantiomers, is not yet easily accessible.



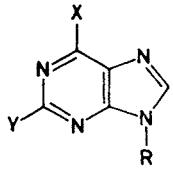
I



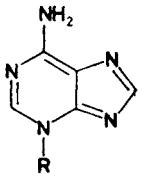
II

As the most suitable procedure we have chosen the three-step reaction sequence consisting in: (i) synthesis of N-(2,3-dihydroxypropyl) derivatives *II* by alkylation

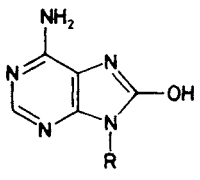
of the heterocyclic base with easily available synthons of the (*RS*)- or (*R*)-glycerol series (*III*), or by subsequent modification of the heterocyclic base in other compounds *II*, (ii) esterification of the 3'-hydroxyl functionality in these compounds with chloromethylphosphonic acid, and (iii) intramolecular cyclization of the formed esters to



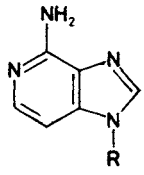
II a, X = NH_2; Y = H
II c, X = NH_2; Y = CH_3
II d, X = NH_2; Y = SCH_3
II e, X = Y = NH_2
II f, X = NH_2; Y = OH
II g, X = $N(CH_3)_2$; Y = OH
II i, X = H; Y = NH_2
II j, X = OH; Y = H
II k, X = $NHNH_2$; Y = H
II l, X = NHOH; Y = H
II m, X = SH; Y = H
II n, X = OH; Y = NH_2



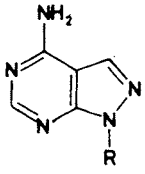
II b



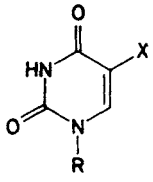
II h



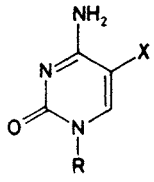
II p



II q



II r, X = H
II s, X = CH_3
II v, X = F

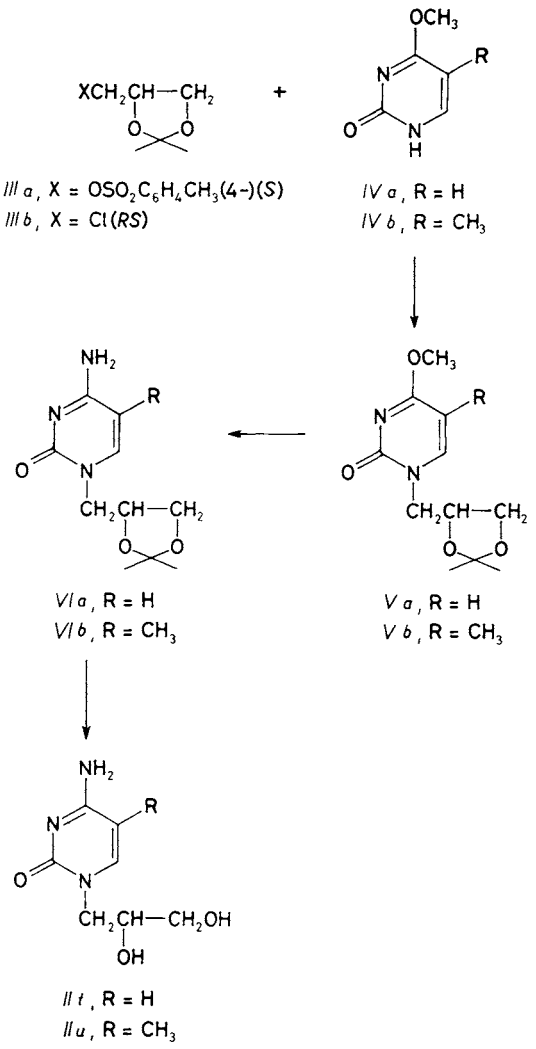


II t, X = H
II u, X = CH_3

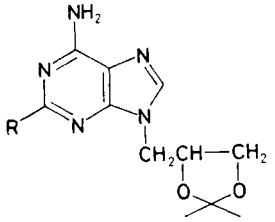
In formula *II*: R = (*RS*)- or (*S*)−$CH_2CH(OH)CH_2OH$ residue

2'-O-phosphonylmethyl ethers of compounds *II* (this reaction which is extraordinarily easy with aqueous alkali metal hydroxides, has been described in detail in our previous communication[1]).

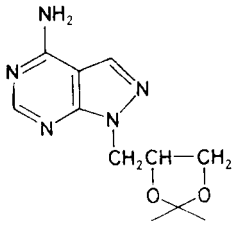
Most of the starting compounds *II* have been prepared by us previously[10-14]. In this study we describe the synthesis, or an improved preparation, of several other derivatives of this series. 1-(*S*)-(2,3-Dihydroxypropyl)cytosine (*IIt*) and 1-(*S*)-(2,3-dihydroxypropyl)-5-methylcytosine (*IIu*) were prepared by condensation of 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-(*R*)-glycerol (*IIIa*, ref.[10]) with 4-methoxy-2-pyrimidone (*IVa*) and its 5-methyl derivative (*IVb*), respectively. The starting compounds *IV* are easily available from the corresponding 2,4-dimethoxypyrimidines



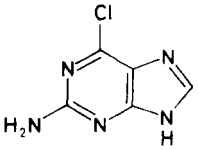
SCHEME 1



VII a, R = NH_2
VII b, R = CH_3

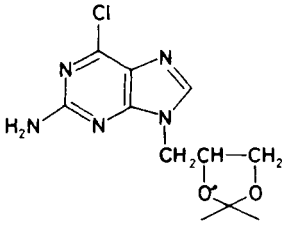


VIII

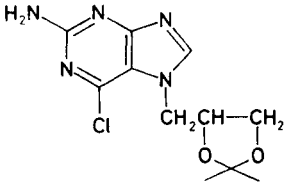


IX

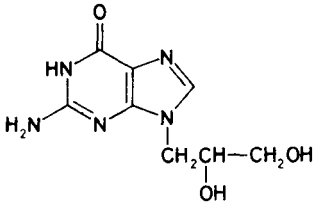
III a



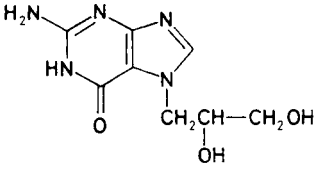
X



XI



II n

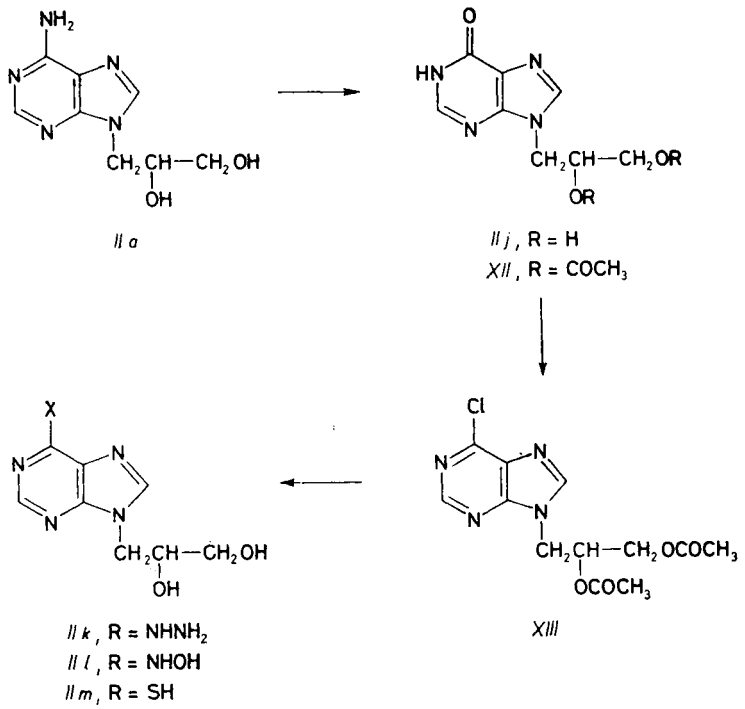


II o

SCHEME 2

and acetyl chloride[15]. This condensation is performed with sodium salt of compounds *IV* in dimethylformamide and leads to isopropylidene derivatives *V* which on reaction with methanolic ammonia are quantitatively converted into the desired protected cytosine derivatives *VI*. Acid hydrolysis of compounds *VI* affords the final (*S*)-(2,3-dihydroxypropyl) derivatives *II* (Scheme 1).

The hitherto undescribed 9-(*S*)-(2,3-dihydroxypropyl)-2,6-diaminopurine (*IIe*) was prepared in the same manner, i.e. by condensation of compound *IIIa* with sodium salt of 2,6-diaminopurine followed by acid hydrolysis of the isolated isopropylidene derivative *VII*. The previously described[11] synthesis of guanine derivative *IIn* is not suitable for preparation of larger quantities because the condensation product is obtained in a low overall yield and the synthesis leads to a mixture of N-7 and N-9 isomers that have to be separated. Therefore, we prepared compound (*S*)-*IIn* by alkylation of sodium salt of 2-amino-6-chloropurine *(IX)* with synthon *IIIa* which gives a high overall yield and affords the protected 9-alkyl derivative *X* as the principal product. This compound can easily be separated from the minor 7-isomer *XI* by chromatography on silica gel. Acid hydrolysis not only removes the isopropylidene protecting group but also simultaneously cleaves the C—Cl bond on the heterocyclic

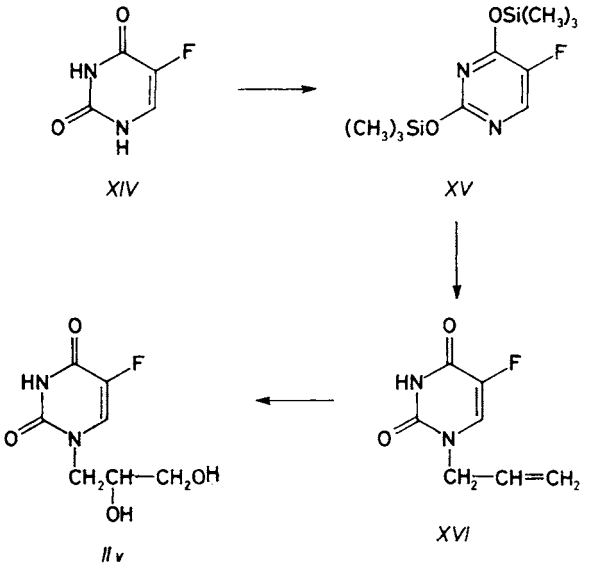


SCHEME 3

base[16], giving rise to the desired guanine derivative *IIn* (or its 7-isomer *IIo*) (Scheme 2).

Of racemic derivatives *II* we newly prepared 9-(*RS*)-(2,3-dihydroxypropyl)-2--methyladenine (*IIc*) and 7-(*RS*)-(2,3-dihydroxypropyl)-4-aminopyrazolo[3,4-d]pyrimidine (*IIq*) by reaction of sodium salt of the corresponding base with 4-chloromethyl-2,2-dimethyl-1,3-dioxolane[11] (*IIIb*) followed by acid hydrolysis of the protected intermediate *VIIb* and *VIII*, respectively. 9-Alkyl derivatives of 6-substituted purines (*IIk*, *IIl*, *IIm*) were obtained from the easily accessible[11] 9-(*RS*)--(2,3-dihydroxypropyl)adenine (*IIa*): deamination with 3-methylbutyl nitrite afforded the hypoxanthine derivative *IIj* which was converted into the 2,3-di-O-acetyl derivative *XII* and further (on treatment with thionyl chloride) into the 6-chloropurine compound *XIII*. This intermediate was heated with hydroxylamine or hydrazine to give the respective 6-hydroxylaminopurine (*IIl*) and 6-hydrazinopurine (*IIk*) derivatives. Reaction of compound *XIII* with thiourea, followed by methanolysis, afforded 9-(*RS*)-(2,3-dihydroxypropyl)-6-thiopurine (*IIm*, cf. ref.[11]) (Scheme 3).

The so far undescribed 1-(*RS*)-(2,3-dihydroxypropyl)-5-fluorouracil (*IIv*) was obtained from 5-fluorouracil (*XIV*) using the silyl variant of the reaction, first employed by us in the synthesis of N-(2,3-dihydroxypropyl)thymine (*IIs*, ref.[15]). Compound *XIV* was converted into the 2,4-bis(trimethoxysilyl) derivative *XV* which, without isolation, was regiospecifically transformed into 1-allyl-5-fluorouracil (*XVI*) by reaction with allyl bromide. This compound reacted with sodium chlorate in the presence of osmium tetroxide to give the desired product *IIv* (Scheme 4).



SCHEME 4

Mixtures of isomeric 2'- and 3'-O-phosphonylmethyl compounds *XX* and *XXI* (derived from racemic compounds *II*), were prepared using the mentioned[1] reaction of compounds *II* with chloromethylphosphonyl dichloride (*XVII*) in triethyl phosphate which proceeds regiospecifically on the vicinal diol grouping of the side-chain. The intermediate chloromethanephosphonic acid ester chlorides, which are formed in practically quantitative yields in most cases, were precipitated with ether from the reaction mixture. Their hydrolysis in neutral medium gives an approximately 1 : 1 mixture of compounds *XVIII* and *XIX* (Table I). As we have observed previously[1], the 2'-ester *XVIII* undergoes acid-catalyzed isomerization to the 3'-isomer *XIX* required for the preparation of the desired isomer *XXI*. It appeared that the men-

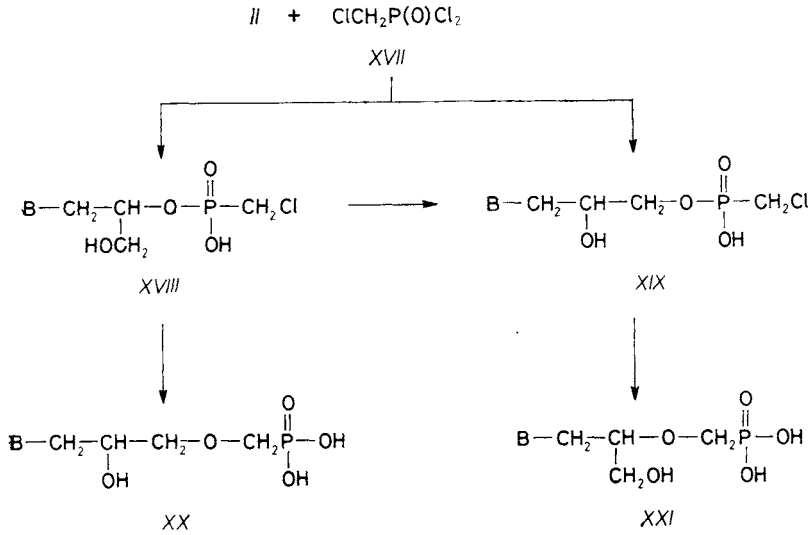
TABLE I

Characteristics of chloromethylphosphonates *XVIII*, *XIX* prepared by the reaction of compound *II* with the dichloride *XVII*

Base	E_{Up} (*XVIII*, *XIX*)	k^a			*XIX*, %[b]	
		S	*XVIII*	*XIX*	τ_0^c	τ_∞^d
a	0·45	7	5·54	7·35	30·2	74·3
b	0·45	7	3·10	4·83	40·4	74·2
c	0·43	8	5·50	6·40	45·0	70·0
d	0·35	9	9·90	11·10	46·0	85·0
e	0·34	7	3·29	4·10	44·7	78·3
g	0·61	9	6·55	7·55	50·0	75·7
h	0·41	8	1·69	2·34	42·6	70·5
i	0·41	7	2·16	2·68	44·3	70·2
j	0·52	7	2·30	4·35	45·3	75·0
k	0·49	6	2·02	4·05	47·0	78·0
l	0·42	6	4·30	5·88	48·3	76·0
m	0·67	7	2·55	4·76	46·2	74·0
n	0·46	7	2·50	2·88	46·8	76·8
o	0·46	7	2·30	2·62	48·2	82·7
p	0·43		—	—	--	76·7
q	0·47	7	6·10	7·42	45·4	74·0
r	0·50	7	0·80	1·41	45·6	72·0
s	0·50	8	0·96	1·20	46·4	71·0
t	0·38	6	1·93	2·69	42·0	—
u	0·35	7	0·51	0·68	47·0	68·6
v	0·88	6	0·90	1·05	43·4	76·0

[a] k $(t_r - t_0)/t_0$, t_r retention time, t_0 hold-up time; [b] content of the 3'-isomer *XIX* formed by the reaction (τ_0) or by the isomerisation (equilibrium mixture, τ_∞); [c] average value, 44·7 ± 4·0%; [d] average value, 75·3 ± 4·0%.

tioned crude ester chloride on mere dissolution in water and subsequent boiling of the resulting solution (containing compounds *XVIII*, *XIX* and hydrogen chloride, formed by hydrolysis of the ester chloride) afforded an equilibrium mixture of both isomers in which the desired isomer *XIX* markedly predominated (>70%). This mixture can be either deionized (e.g., on Dowex 50 in the H^+-form) with isolation of compounds *XVIII* and *XIX*, or used directly (without isolation) in the next reaction step. This consists in treatment with an alkali metal hydroxide (usually aqueous solution of sodium hydroxide at elevated temperature) which during several hours converts quantitatively the mentioned compounds into a mixture of isomeric racemic O-phosphonylmethyl derivatives *XX* and *XXI*, in which the 2'-isomer *XXI* (analog of HPMPA) predominates (>70%). The reaction can be easily followed either by HPLC or paper electrophoresis. The reaction products are (in some cases after deionization) isolated as free acids by chromatography on an ion-exchanger, or are converted into salts (preferably into sodium salts) (Scheme 5, Table II).



SCHEME 5

In certain cases, the isomers *XX* and *XXI* can be distinguished by HPLC, however, the differences are too small to allow their preparative separation. Also, the attempted separation on cation- or anion-exchanging resins was not successful. On the other hand, chloromethylphosphonates *XVIII* and *XIX* – in some cases (e.g. compounds *XVIIIe* and *XIXe* or *XIIIp* and *XIXp*) – may be separated by chromatography on Dowex 50 (H^+-form) or by preparative reversed-phase HPLC (e.g. compounds

TABLE II

Characteristics of compounds *XX*, *XXI*

Base	Yield %	R_F (S4)	E_{Up}	HPLC S	HPLC k^a	Formula (M.w.)	Calculated/Found % N	Calculated/Found % P
a	—	0·22	0·75	6	1·82	cf.[1]		cf.[1]
b	64	0·22	0·75	5	1·53	$C_9H_{12}N_5Na_2O_5P.$ .2 H_2O (383·3)	18·27 18·61	8·10 8·15
c	55	0·27	0·67	9	1·25	$C_{10}H_{14}N_5Na_2O_5P.$ .2.H_2O (397·3)	17·63 17·47	7·81 7·78
d	72	0·32	0·66	9	4·10	$C_{10}H_{14}N_5O_5PSNa_2$ (393·4)[b]	17·81 17·55	7·89 8·08
e	70	0·12	0·63	7	0·87	$C_9H_{13}N_6O_5PNa_2.$ .H_2O (380·3)	22·10 21·89	8·16 7·98
f	—	0·14	0·65	7	0·76	$C_9H_{12}N_5O_6PNa_2$ .H_2O (381·3)	18·37 18·04	8·14 8·38
g	66	0·43	0·62	9	5·87	$C_{11}H_{16}N_5O_5PNa_2$ (375·4)	18·66 18·95	8·27 8·36
h	70	0·18	0·87	7	4·49	$C_9H_{12}N_5O_6PNa_2.$ .H_2O (381·3)	18·37 18·52	8·14 8·41
i	72	0·23	0·74	5	0·77	$C_9H_{12}N_5O_5PNa_2.$ .H_2O (365·3)	19·18 18·80	8·50 8·52
j	63	0·24	0·79	6	3·23	$C_9H_{13}N_4O_6P$ (304·3)[c]	18·42 18·75	10·20 9·93
k	55	0·21	0·78	6	1·45	$C_9H_{13}N_6O_5PNa_2.$ .H_2O (380·3)	22·10 21·93	8·16 8·34
l	78	0·23	0·84	6	1·38	$C_9H_{12}N_5O_6PNa_2.$ .H_2O (381·3)	18·37 18·61	8·14 8·25
m	70	0·15	1·00	6	3·20	$C_9H_{11}N_4O_5PSNa_2.$ .H_2O (382·3)[d]	14·66 14·38	8·20 7·91
n	71	0·08	0·85	5	1·42	$C_9H_{12}N_5Na_2O_6.$ .H_2O (381·3)	18·37 18·10	8·14 8·15
o	57	0·16	0·75	5	2·58	$C_9H_{12}N_5Na_2O_6P.$ .H_2O (381·3)	18·37 18·38	8·14 8·01
p	—	0·23	0·71	7	1·40	$C_{10}H_{13}N_4O_5P.$ .3H_2O (354·3)	15·80 15·71	8·75 9·18
q	79	0·25	0·78	6	2·12	$C_9H_{12}N_5Na_2O_5.$ .2H_2O (383·3)	18·27 17·97	8·10 8·35

TABLE II
(*Continued*)

Base	Yield %	R_F (S4)	E_{Up}	HPLC S	HPLC k^a	Formula (M.w.)	Calculated/Found % N	Calculated/Found % P
r	75	0·21	0·86	6	2·06	$C_8H_{11}N_2Na_2O_7P.$ $.3H_2O$ (378·3)	7·41 7·23	8·21 8·52
s	70	0·28	0·80	6	2·50	$C_9H_{14}N_2Na_2O_7P.$ $.H_2O$ (357·3)	7·84 7·49	1·69 9:01
t	20	0·16	0·82	5	4·22	$C_8H_{12}N_3Na_2O_6P$ (323·3)	13·00 12·95	9·60 9·42
u	18	0·30	0·78	5	5·12	$C_9H_{14}N_3Na_2O_6P.$ $.2H_2O$ (373·3)	11·26 10·98	8·31 8·66
v	59	0·15	1·08	5	1·22	$C_8H_{10}FLi_2N_2O_7P$ (310·1)[e]	9·04 9·21	10·01 9·84

[a] $k = (t_r - t_0)/t_0$, t_r retention time, t_0 hold-up time; [b] calculated: 8·15% S. found: 8·43% S; [c] calculated: 35·52% C, 4·30% H, found: 35·50% C, 4·19% H; [d] calculated: 8·39% S, found: 8·31% S; [e] calculated: 6·13% F, found: 6·32% F.

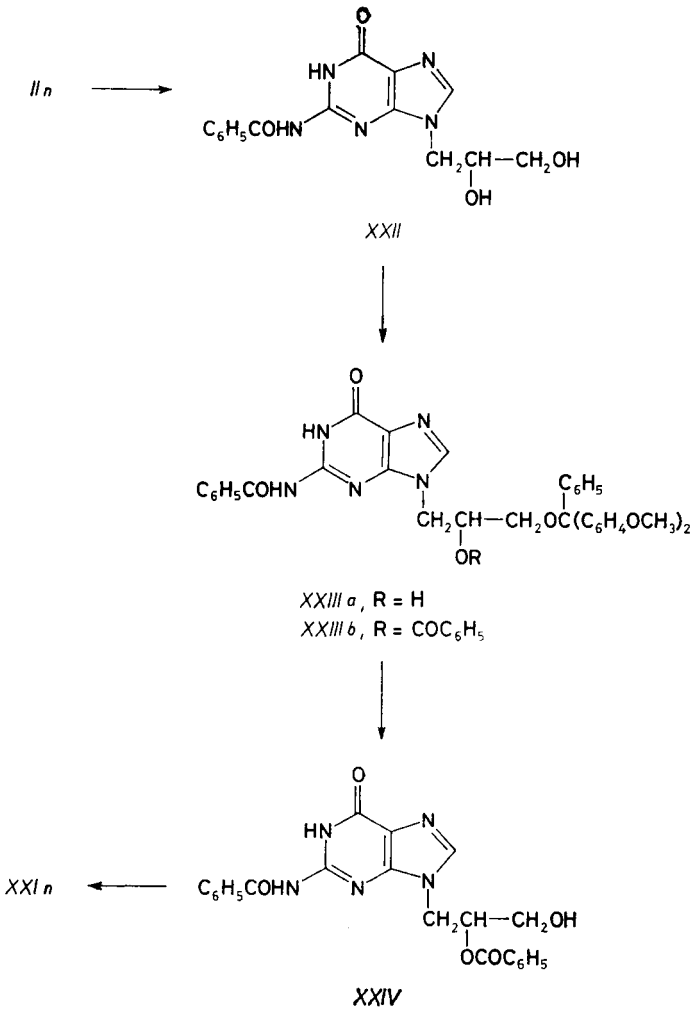
XVIIIt and *XIXt* or *XVIIIu* and *XIXu*). The thus-isolated compounds *XIX* are then transformed into the 2'-phosphonylmethyl derivatives *XXI*.

In the case of cytosine and 5-methylcytosine derivatives the last reaction step (alkali treatment) is accompanied by an extensive deamination which results in the formation of uracil (*XXr*, *XXIr*) and thymine (*XXs*, *XXIs*) derivatives. Thus, this reaction of the above-mentioned isomers *XIXt* and *XXIu* (separated by HPLC) allowed us to obtain simultaneously the pure racemates of *XXIr*, *XXIs*, *XXIt* and *XXIu*. Modification of the heterocyclic base was utilized also in other cases: deamination of the adenine derivative *I* or *IIa* with 3-methylbutyl nitrite afforded the hypoxanthine derivative (*S*)-*XXIj* or a racemic mixture of *XXj* and *XXIj*, whereas an analogous selective deamination of the 2,6-diaminopurine derivatives *XXe* and *XXIa* in position 2 gave the racemic 2-hydroxyadenine derivatives *XXf* and *XXIf*. Interestingly, with heterocyclic bases, expected to be labile toward strong alkali (e.g., 6-thiopurine or 5-fluorouracil derivatives), no significant formation of side-products (as the result of the expected degradation) was observed.

The thus-obtained compounds were characterized by usual methods: structure of the heterocyclic base (and identity of the N-isomers) was proven by UV spectra, homogeneity of the products was checked by chromatographic methods and electro-

phoresis (the mobility of compounds *XVIII* and *XIX* corresponds to dissociation to the first degree, that of compound *XX* and *XXI* to the second degree, in a weakly alkaline medium). Pure isomers *XXI* were also investigated using the NMR spectroscopy.

The prepared series of compounds (Table III) was subjected to preliminary antiviral and other biological activities[3-5,17]. On the basis of these data, we synthesized in the next phase of our investigation several pure (*S*)-2'-isomers *XXI* with the most promising activity: derivative of 2,6-diaminopurine, (*S*)-*XXIe* (HPMPDAP), 3--deazaadenine, (*S*)-*XXIp*, guanine, (*S*)-*XXIn* (HPMPG), and cytosine, (*S*)-*XXIt*



SCHEME 6

(HPMPC). Compound (*S*)-*XXIe* was synthesized using the method described in Scheme 5: isomers *XVIIIe* and *XIXa* were prepared from 9-(*S*)-(2,3-dihydroxypropyl)-2,6-diaminopurine ((*S*)-*IIe*). They can be separated to a large extent directly after the isomerization by crystallization from water and are well separable by chromatography on Dowex 50. The isomer *XIXe* was converted into compound (*S*)-*XXIe* by reaction with sodium hydroxide and, after deionization, the product was isolated by chromatography on an anion-exchanger. The same procedure was applied to the synthesis of the 3'-isomer of 3-deaza derivative (*S*)-*XIXp* which was isolated by chromatography on Amberlite IRC 50 (H^+-form).

In analogy to the synthesis[1] of HPMPA (*I*), 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)guanine ((*S*)-*XXIn*) and the corresponding cytosine derivative (*S*)-*XXIt* were prepared by regiospecific synthesis from the corresponding N-(*S*)-(3-hydroxy-2-benzoyloxypropyl) derivatives *XXIV* and *XXVII* which contain only one free hydroxy group, in position 3 of the side-chain. Their reaction with chloride *XVII* can thus afford only the 3'-chloromethanephosphonate (and the subsequent alkali treatment only the 2'-isomer *XXI*). Guanine and cytosine contain basic amino group which should be protected to enable an unequivocal selective benzoylation of the 2'-hydroxyl functionality in the starting compound *II*. To simplify the situation, we have also used N-benzoyl protection for this purpose. It was introduced by succesive treatment of compounds *II* in pyridine with chlorotrimethylsilane and with benzoyl chloride[18], followed by controlled partial debenzoylation of the originally formed N,N-dibenzoyl derivative. In this way, compound (*S*)-*IIn* (vide supra) afforded the N^2-benzoyl derivative *XXII*, characterized by ^{1}H NMR spectrum.

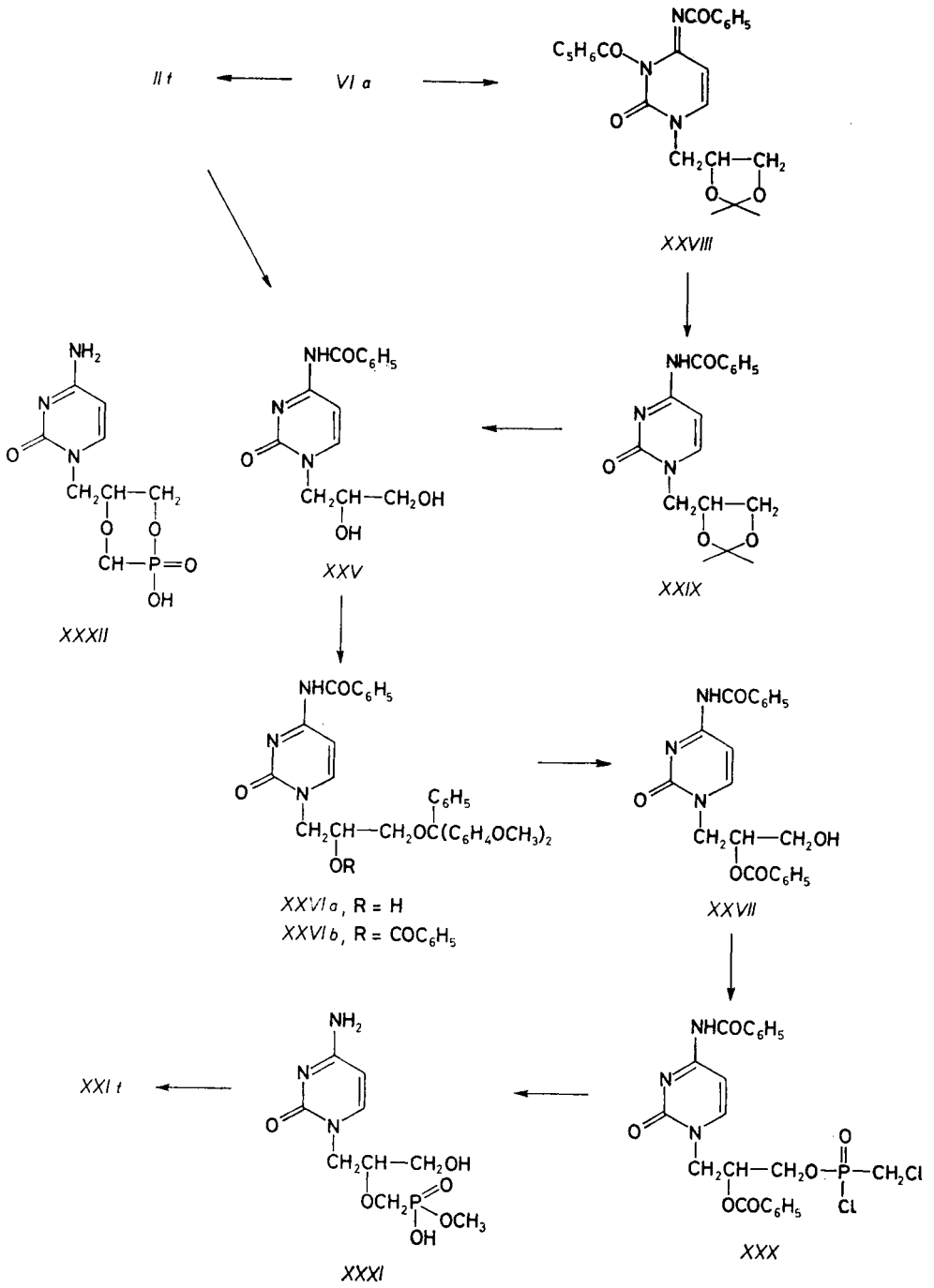
TABLE III
Modifications of base in compounds of the type *I*

Compound	Base residue	Compound	Base residue
a	Adenin-9-yl	*m*	6-Thiopurin-9-yl
b	Adenin-3-yl	*n*	Guanin-9-yl
c	2-Methyladenin-9-yl	*o*	Guanin-7-yl
d	2-Methylthioadenin-9-yl	*p*	3-Deazaadenin-9-yl
e	2,6-Diaminopurin-9-yl	*q*	4-Aminopyrazolo[3,4-*d*]-pyrimidin-7-yl
f	2-Hydroxyadenin-9-yl		
g	N^6-Dimethyladenin-9-yl	*r*	Uracil-1-yl
h	8-Hydroxyadenin-9-yl	*s*	Thymin-1-yl
i	2-Aminopurin-9-yl	*t*	Cytosin-1-yl
j	Hypoxanthin-9-yl	*u*	5-Methylcytosin-1-yl
k	6-Hydrazinopurin-9-yl	*v*	5-Fluorouracil-1-yl
l	6-Hydroxylaminopurin-9-yl		

Reaction of *XXII* with dimethoxytrityl chloride afforded 3'-O-dimethoxytrityl derivative *XXIIIa* which was benzoylated to give compound *XXIIIb*; because of lability of the intermediate *XXIIIa*, the benzoylation was performed by base-catalyzed reaction with benzoyl cyanide in acetonitrile[19]. The obtained fully protected intermediate was without purification cleaved with trifluoroacetic acid and the resulting 2,2'-dibenzoyl derivative *XXIV* was isolated by chromatography. The structure of this compound was confirmed by ^{1}H NMR spectrum. Its reaction with chloride *XVII* in triethyl phosphate, followed by hydrolysis of the crude reaction product with aqueous sodium hydroxide, afforded isomerically pure compound (*S*)-*XXIn* (Scheme 6).

An analogous procedure was employed for the preparation of 2',4-dibenzoyl derivative of compound (*S*)-*IIt* (*XXVII*): the starting compound (prepared as described above) was first converted by the above technique into its N^4-benzoyl derivative *XXV* which was dimethoxytritylated to give compound *XXVIa*. Its benzoylation with benzoyl cyanide afforded the intermediate *XXVIb* which on detritylation with dilute trifluoroacetic acid yielded the key intermediate *XXVII*. The structure of this compound was also confirmed by ^{1}H NMR spectrum. For the synthesis of compound *XXV* we investigated still another method, starting from 2,3-O-isopropylidene derivative *VIa*, an intermediate in the preparation of compound (*S*)-*IIt* (see Scheme 1): benzoylation with benzoyl chloride afforded N-dibenzoyl derivative *XXVIII* which was ammonolyzed to give the N^4-benzoyl derivative *XXIX* and further acid-hydrolyzed to afford the above-mentioned compound *XXV*. Although this procedure circumvents the acid hydrolysis of compound *XXVIII* to compound (*S*)-*IIt* as well as the use of chlorotrimethylsilane, from the preparative standpoint it offers no considerable advantage compared with the above-described synthesis of compound *XXV*.

Treatment of compound *XXVII* with chloromethylphosphonyl dichloride (*XVII*), followed by precipitation of the reaction mixture with ether, affords smoothly the ester chloride *XXX*. As already mentioned, the reaction of chloromethylphosphonates, derived from compound *IIt* (*XVIIIt*, *XIXt*), with aqueous alkali metal hydroxide is accompanied with deamination to the uracil derivatives *XXr* and *XXIr*. To prevent loss of the product due to this undesired reaction, the intermediate *XXX* was first hydrolyzed in a neutral medium, dried and treated with boiling solution of sodium methoxide in methanol. This reaction results in N,O-debenzoylation and subsequent transformation of the formed chloromethylphosphonate (*S*)-*XIXt* into methyl ester of (*S*)-*XXIt* (compound *XXXI*). This reaction proceeds via the cyclic intermediate (methyl ester of the cyclic phosphonate *XXXII*) which is opened by the methoxide anion[20]. The crude reaction mixture was then treated with bromotrimethylsilane to remove the methyl ester group (for obvious reasons the alkaline hydrolysis, very easy with esters of this type, cannot be used). After deionization, the obtained product (*S*)-*XXXIt* was isolated by chromatography on Dowex 1.


II t
VI a
XXVIII
XXIX
XXV
XXXII
XXVI a, R = H
XXVI b, R = COC6H5
XXVII
XXX
XXXI
XXI t

SCHEME 7

As a side-product of this reaction we obtained the cyclic phosphonate *XXXII*, arising by concurrent hydrolysis of the mentioned cyclic intermediate. Both the compounds can be well separated in the isolation step (Scheme 7).

The ester chloride *XXX* cannot be methanolyzed directly, without previous hydrolysis. In such case, the neutral mixed methyl ester of chloromethylphosphonic acid, formed as an intermediate, easily migrates to form a 1 : 1 mixture of 2'- and 3'-isomers which in turn give rise to a mixture of methyl esters of compounds (*S*)-*XXIb* and (*S*)-*XXb*. Such a participation does not occur with the less reactive protected 3'-O--chloromethylphosphonate formed by hydrolysis of compound *XXX*.

The present communication summarizes our methodical experience on the syntheses of HPMP-derivatives, some of which attract attention as potential antivirals. The methods starting from N-(*S*)-(2,3-dihydroxypropyl) derivatives of heterocyclic bases, pay special attention to compounds with the most significant antiviral effect: derivatives of adenine (HPMPA, *I*, see ref.[1]), 2,6-diaminopurine (HPMPDAP, *XXIe*), guanine (HPMPG, *XXIn*), 3-deazaadenine (*XXIp*) and cytosine (HPMPC, *XXIt*). In all cases we also elaborated effective syntheses of the starting compounds *II* using the chiral synthon *III*, easily obtained from D-mannitol[10]. These methods make the corresponding derivatives (*S*)-*IIa*, *IIe*, *IIn* and *IIt* well accessible (in the case of *IIn* the availability of 2-amino-6-chloropurine represents a limiting factor). The introduction of the O-phosphonylmethyl ether functionality by intramolecular cyclization of chloromethylphosphonates of compounds *II*, prepared by reaction of free or specifically protected compounds (*S*)-*II* with the commercially available chloromethylphosphonyl dichloride (*XVII*), seems to be superior to the direct nucleophilic substitution of the organophosphorus synthon (e.g. diester of *p*-toluenesulfonyloxymethylphosphonic acid[1] or methanesulfonyloxymethylphosphonic acid[21]) by anions generated from the hydroxyl groups of free or specifically protected compounds *II*. The simplest variant of the reaction, i.e. the reaction of unprotected compounds (*S*)-*II* according to Scheme 5, can be applied to the preparation of the desired homogeneous 2'-isomer (*S*)-*XXI* mainly in those cases when the isomers (*S*)-*XVIII* and (*S*)-*XIX* are well separable, e.g. by ion-exchanger chromatography or crystallization (compound (*S*)-*XXIe* etc.). The use of preparative reversed-phase chromatography is generally applicable to all the cases required; the effectivity of the described reaction is emphasized by the possibility of substantial enrichment of the chloromethylphosphonate mixture with the key isomer *XIX*, and recycling the 3'-isomer *XVIII*, separated as the side-product. As seen from Table I, both the original composition of the mixture of isomeric chloromethylphosphonates (45 ± 4% of the 3'-isomer), arising by reaction of compounds *II* with chloride *XVII*, and the equilibrium composition of this mixture after isomerization (75 ± 4% of the 3'-isomer) are completely independent of the character of the heterocyclic base in compounds *II* (purine or pyrimidine derivatives of basic, neutral or acidic character). This method (which starts with the very facile preparation of racemic compounds *II*) is also the

most suitable one for a rapid evaluation of biological activity of additional novel analogs of compound *I* with modified heterocyclic base.

The second variant which is based on preceding selective protection by benzoylation of the hydroxyl in position 2′ of the side-chain in compounds (*S*)-*II*, has been generalized in this work. Although it requires a multistep preparation of compounds *XXIV* or *XXVIII*, the individual steps proceed practically quantitatively and the intermediates need not be isolated in the pure state. A certain limitation of this method is the difficult availability of dimethoxytrityl chloride and manipulation with benzoyl cyanide (which, however, could be replaced by other benzoylating reagents, applied in solvents other than pyridine, e.g. N-benzoylimidazole). Alkaline hydrolysis or methanolysis of the intermediates leading to the final reaction products is accompanied by simultaneous removal of the benzoyl protecting groups. This variant could be improved by use of pre-protected (N-benzoylated) heterocyclic bases already in the preparation of compounds *II* which would make the first (and most inefficient) step in the preparation of specifically protected key compounds unnecessary. The applicability of all the above variants was verified by syntheses of the described compounds in quantities sufficient for biological assays on animal models of virus infections[22].

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated 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) was performed on Silufol UV 254 plates (Kavalier, Votice, Czechoslovakia) in the systems S1 chloroform–methanol (19 : 1), S2 chloroform–methanol (9 : 1) and S3 chloroform–methanol (4 : 1). Paper chromatography was carried out on a Whatman No 1 paper in the system S4 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Paper electrophoresis was done on a Whatman No 3 MM paper in buffer S5 (triethylammonium hydrogen carbonate), concentration 0·1 mol l^{-1}, at 40 V cm^{-1}. Liquid chromatography (HPLC) was performed on columns (250 × 4 mm) of C18-silica gel Separon SGX (10 μm) in 0·05M buffer S5, containing the following amount (vol.%) of methanol: 2·5% (S6), 5% (S7), 10% (S8), 15% (S9); elution rate 1 ml min^{-1}, detection at 254 nm.

UV absorption spectra (λ_{max} in nm (ε)) were determined on a PU 8800 UV/VIS spectrophotometer (Pye Unicam, Cambridge, Great Britain), NMR spectra on a Varian XL-200 instrument (chemical shifts in ppm, coupling constants in Hz). Preparative chromatography was performed on columns of silica gel according to Pitra (30–40 μm) or on loose layers (40 × 16 × × 0·4 cm) of silica gel, containing indicator (both products of Service Laboratories of this Institute).

Dimethylformamide and acetonitrile (Janssen, Belgium) were dried over phosphorus pentoxide, distilled in vacuo and stored over molecular sieves. Bromotrimethylsilane, benzoyl cyanide, triethyl phosphate, 4-dimethylaminopyridine and trifluoroacetic acid were Janssen (Belgium) products, 4-aminopyrazolo[3,4-*d*]-pyrimidine, 2-methyladenine and sodium chlorate were purchased from Lachema (Czechoslovakia), and 2-amino-6-chloroguanine from Mack (F.R.G.). 2,6-Diaminopurine was prepared[23] from its hemisulfate (Sigma, U.S.A.), 5-fluorouracil was

a Hofmann La Roche product, allyl bromide, hexamethyldisilazane and osmium tetroxide were obtained from Merck (F.R.G.), chloromethylphosphonyl dichloride from Alfa (U.S.A.). 4--Methoxy-2-pyrimidone and 4-methoxy-5-methyl-2-pyrimidone were prepared according to ref.[15], 3-deazaadenine according to ref.[14]. The preparation of chlorobis(*p*-methoxyphenyl)-phenylmethane (dimethoxytrityl chloride) followed that described in ref.[24]. The following compounds (*RS*)- and (*S*)-*II* were prepared as described previously: *IIa*, *IIr*, *IIs*, *IIt* (ref.[10]), *IIb*, *IIc*, *IId*, *IIg*, *IIj*, *IIn*, *IIo* (ref.[11]), *IIh* (ref.[13]), *IIi* (ref.[12]) and *IIp* (ref.[14]).

9-(*S*)-(2,3-Dihydroxypropyl)-2,6-diaminopurine ((*S*)-*IIe*)

A mixture of 2,6-diaminopurine (12·0 g, 80 mmol) and sodium hydride (1·92 g, 80 mmol; prepared by washing a 60% dispersion of NaH in paraffin with light petroleum) in dimethylformamide (150 ml) was stirred at 80°C for 1 h, a solution of compound *IIIa* (87 mmol) in dimethylformamide (50 ml) was added and the mixture was stirred at 100°C for 16 h under exclusion of moisture. After evaporation at 40°C/13 Pa, the residue was crystallized from methanol, the mother liquor was taken down in vacuo and the residue was again crystallized under the same conditions. The total yield was 10·6 g (50%) of compound (*S*)-*VIIa*, not melting up to 300°C. For $C_{11}H_{16}N_6O_2$ (264·3) calculated: 49·98% C, 6·10% H, 31·80% N; found: 49·59% C, 6·06% H, 31·87% N. R_F 0·60 (S3).

A mixture of compound (*S*)-*VIIa* (10·5 g, 40 mmol) and 0·25M sulfuric acid (500 ml) was stirred to homogeneity and then set aside at room temperature for 24 h. The mixture was neutralized with saturated barium hydroxide solution, taken to the boil and filtered through Celite while hot. The Celite was washed with boiling water (2 l), the filtrate was taken down in vacuo, the residue was codistilled with ethanol, mixed with ethanol–ether (1 : 1), filtered, washed with ether and dried to give 9·7 g (100%) of compound (*S*)-*IIe*, m.p. 229—230°C. For $C_8H_{12}N_6O_2$ (224·2) calculated: 42·85% C, 5·40% H, 37·49% N; found: 43·25% C, 5·27% H, 37·39% N; $[\alpha]_D^{20}$ −37·5° (*c* 0·5, 0·1M-HCl).

9-(*S*)-(2,3-Dihydroxypropyl)guanine ((*S*)-*IIn*) and
7-(*S*)-(2,3-Dihydroxypropyl)guanine ((*S*)-*IIo*)

2-Amino-6-chloropurine (*IX*, 12·8 g, 75 mmol) was added to a suspension of sodium hydride (1·8 g, 75 mmol) in dimethylformamide (250 ml) and the mixture was stirred for 1 h under exclusion of moisture (calcium chloride tube). A solution of compound *IIIa* (25·3 g, 88·5 mmol) in dimethylformamide (50 ml) was added and the mixture was heated to 100°C for 14 h. After evaporation at 40°C/2 kPa, the residue was codistilled with toluene (2 × 100 ml) and extracted with boiling chloroform (1 l total). The solvent was evaporated and the residue chromatographed on a column of silica gel (400 ml) in chloroform. The chromatography afforded two fractions: the first (after crystallization from ethanol–light petroleum) afforded 8·55 g (40%) of compound *X*, m.p. 204—206°C. For $C_{11}H_{14}ClN_5O_2$ (283·7) calculated: 46·56% C, 4·97% H, 12·50% Cl, 24·69% N; found: 46·48% C, 4·83% H, 12·74% Cl, 24·92% N. R_F 0·53 (S2). UV (methanol): 247 (6 700), 308 (8 500), 265 (min). $[\alpha]_D^{20}$ −9·7° (*c* 0·5, dimethylformamide).

The second fraction on crystallization from ethanol–light petroleum gave 2·62 g (12·3%) of compound *XI*, m.p. 185°C. For $C_{11}H_{14}ClN_5O_2$ (283·7) calculated: 46·56% C, 4·97% H, 12·50% Cl, 24·69% N; found: 46·39% C, 4·77% H, 12·46% Cl, 24·81% N. R_F 0·40 (S2). UV (methanol): 222·5 (18 300), 321 (4 800), 274 (min). $[\alpha]_D^{20}$ −48·2° (*c* 0·5, dimethylformamide).

A suspension of compound *X* (8 g, 28·2 mmol) in 1M hydrochloric acid (300 ml) was stirred and heated to 100°C for 2 h under reflux condenser. After cooling, the mixture was made alkaline

with ammonia and taken down in vacuo. The residue was dissolved in boiling water, mixed with the same volume of water and left to crystallize in a refrigerator overnight. The product was collected on filter, washed with water, acetone, ether, and dried in vacuo. Yield 5·8 g (91%) of compound *IIn*, not melting up to 300°C. For $C_8H_{11}N_5O_3$ (225·2) calculated: 42·66% C, 4·92% H, 31·10% N; found: 42·51% C, 4·80% H, 31·29% N. UV (pH 2): 258 nm (11 500). $[\alpha]_D^{20}$ −39·9° (*c* 0·5, 0·1M HCl).

Compound *XI* (2·3 g, 8 mmol) was treated with 1M hydrochloric acid (100 ml), as described for hydrolysis of compound *X*. Crystallization afforded 1·75 g (96%) of compound *IIo*, not melting up to 300°C. For $C_8H_{11}N_5O_3$ (225·2) calculated: 42·66% C, 4·92% H, 31·10% N; found: 42·50% C, 4·63% H, 30·86% N. $[\alpha]_D^{20}$ −72·3° (*c* 0·5, 0·1M-HCl).

1-(*S*)-(2,3-Dihydroxypropyl)cytosine ((*S*)-*IIt*)

4-Methoxy-2-pyrimidone (*IVa*, 45 g, 0·36 mol) was added to an ice-cold suspension of sodium hydride (8·64 g, 0·36 mol) in dimethylformamide (800 ml). The mixture was stirred for 1 h at 0°C under exclusion of moisture and then for 30 min at 60°C. A solution of compound *IIIa* (107·5 g, 0·38 mol) in dimethylformamide (400 ml) was added at 60°C and the mixture was stirred at this temperature for 16 h. After evaporation at 50°C/2 kPa, the residue was codistilled with toluene (2 × 100 ml) and extracted with boiling chloroform (2 l total). The extract was concentrated in vacuo to approx. 300 ml and applied on a column of silica gel (1 l) in chloroform. The column was eluted with chloroform and the product fraction after evaporation in vacuo crystallized from ether–light petroleum. Yield, 43·7 g (51%) of compound *Va*, m.p. 107°C. For $C_{11}H_{16}N_2O_4$ (240·3) calculated: 54·99% C, 6·71% H, 11·66% N; found: 54·80% C,6·61%H, 11·50% N. R_F 0·55 (S2).

A solution of compound *Va* (43 g, 0·18 mol) in 30% methanolic ammonia (800 ml) was heated to 120°C for 8 h. After evaporation in vacuo the residue was crystallized from ethanol. The mother liquor was concentrated and the residue again crystallized under the same conditions. Total yield 35·4 g (88%) of compound *VIa*, m.p. 274—275°C. For $C_{10}H_{15}N_3O_3$ (225·3) calculated: 53·32% C, 6·71% H, 18·66% N; found: 53·23% C, 6·66% H, 18·61% N. R_F 0·46 (S3). $[\alpha]_D^{20}$ −76·1° (*c* 0·5, 0·1M-HCl).

A mixture of compound *VIa* (35 g, 0·16 mol) and 0·25M sulfuric acid (500 ml) was stirred to dissolution, set aside overnight and neutralized with saturated barium hydroxide solution. The suspension was taken to the boil, filtered through Celite while hot, the Celite was washed with boiling water (2 l) and the filtrates were evaporated in vacuo. The residue in ethanol (300 ml) was refluxed with stirring, cooled, mixed with ether (600 ml) and allowed to crystallize at 0°C overnight to yield 29·3 g (100%) of compound (*S*)-*IIt*, m.p. 170—171°C. For $C_7H_{11}N_3O_3$ (185·2) calculated: 45·40% C, 5·99% H, 22·70% N; found: 45·59% C, 6·02% H, 22·61% N. $[\alpha]_D^{20}$ −92·8° (*c* 0·5, 0·1M-HCl). R_F 0·54 (S4).

1-(*S*)-(2,3-Dihydroxypropyl)-5-methylcytosine ((*S*)-*IIu*)

Sodium hydride (1·92 g, 80 mmol) was added to a solution of 4-methoxy-5-methyl-2-pyrimidone (*IVb*, 11·2 g, 80 mmol) in dimethylformamide (250 ml). After stirring at 60°C for 1 h, a solution of compound *IIIa* (21·8 g, 76 mmol) in dimethylformamide (50 ml) was added. The mixture was heated at 100°C with stirring and exclusion of moisture for 14 h and worked up as described for compound (*S*)-*IIt* (preparation of *Va*). Chromatography on silica gel afforded the product *Vb* (R_F 0·75 in S1) which was heated with 30% methanolic ammonia (300 ml) to 120°C for 14 h. Evaporation in vacuo and crystallization from ethanol (ether added to turbidity) afforded 8·7 g (47%) of compound *VIb*, m.p. 235°C, R_F 0·16 (S2). For $C_{11}H_{17}N_3O_3$ (239·3) calculated:

55·22% C, 7·16% H, 17·57% N; found: 55·56% C, 7·16% H, 17·41% N. ^{1}H NMR ($CDCl_3$): 1·33 s + 1·40 s, 6 H (isopropylidene); 1·93 s, 3 H (5-CH_3, J = 1·0); 3·55–3·80 m, 2 H (3′-CH_2); 4·0–4·20 m, 2 H (1′-CH_2); 4·43 m, 1 H (2′-CH); 4·0–4·20 m, 2 H (1′-CH_2); 4·43 m, 1 H (2′-CH); 6·50 br d, 2 H (NH_2); 7·17 d, 1 H (6-H, J(6,CH_3) = 1·0). $[\alpha]_D^{20}$ −27·3° (*c* 0·5, dimethylformamide).

A solution of compound *VIb* (8·0 g, 33 mmol) in 0·25M sulfuric acid (100 ml) was worked up as described for compound (*S*)-*IIt* (hydrolysis of *VIa*). Evaporation and crystallization from ethanol (ether added to turbidity) afforded 5·9 g (89%) of compound (*S*)-*IIu*, m.p. 182°C. For $C_8H_{13}N_3O_3$ (199·2) calculated: 48·23% C, 6·58% H, 21·10% N; found: 48·34% C, 6·52% H, 21·42% N. ^{1}N NMR ($(CD_3)_2SO$): 1·81 s, 3 H (5-CH_3, J = 1·0); 3·20–4·0 unresolved multiplet, 5 H (1′-CH_2 + 3′-CH_2 + 2′-CH); 4·72 t, 1 H (3′-OH, J = 6·0); 4·94 d, 1 H (2′-OH, J = 5·0); 6·86 br s, 2 H (NH_2); 7·32 d, 1 H (6-H, J(6,CH_3) = 1·0). $[\alpha]_D^{20}$ −71·9° (*c* 0·5, 0·1M-HCl).

9-(*RS*)-(2,3-Dihydroxypropyl)hypoxanthine (*IIj*)

3-Methylbutyl nitrite (60 ml) was added under argon to a solution of hydrate of compound *IIa* (15 g, 66 mmol) in 80% acetic acid (600 ml). The mixture was allowed to stand at room temperature for 2 days in a stoppered bottle. After evaporation in vacuo, the residue was codistilled with water (5 × 100 ml) and, after dissolution in water (100 ml) applied onto a column of Dowex 50 × 8 (H^+-form, 250 ml). Elution with water afforded (with considerable retention) the UV-absorbing fraction of the product. After evaporation, the residue was mixed with ethanol–ether (1 : 1, 200 ml), and filtered to give chromatographically pure compound *IIj* (13·8 g, 92%) identical with an authentic sample[11].

9-(*RS*)-(2,3-Diacetoxypropyl)-6-chloropurine (*XIII*)

A mixture of compound *IIj* (12·8 g, 61 mmol), acetic anhydride (400 ml) and 4-dimethylaminopyridine (2 g) was stirred overnight. After evaporation, the residue was codistilled with toluene (2 × 100 ml) and crystallized from ethanol to give 15·1 g (84%) of chromatographically pure compound *XII* (R_F 0·15 in S3), identical with an authentic sample[11]. This product was stirred and refluxed for 4 h with a mixture of chloroform (220 ml), thionyl chloride (20 ml) and dimethylformamide (1·4 ml). The mixture was poured into a suspension of sodium hydrogen carbonate (64 g) in ice-cold water (160 ml), the chloroform layer was separated, the aqueous one was extracted with chloroform (3 × 100 ml) and the combined organic phases were dried over sodium sulfate. The solvent was evaporated in vacuo and the residue was chromatographed on a column of silica gel (300 ml) in chloroform. The product fractions were combined and taken down in vacuo to afford 14·4 g (90%) of compound *XIII* which was used in further reactions. R_F 0·70 (S3), identical with an authentic sample[11].

9-(*RS*)-(2,3-Dihydroxypropyl)-6-hydrazinopurine (*IIk*)

A mixture of compound *XIII* (2 g, 6·5 mmol) and 96% hydrazine hydrate (30 ml) was heated in an autoclave to 100°C for 8 h. After cooling, the mixture was evaporated, the residue codistilled with water (2 × 50 ml), dissolved in water (50 ml), acidified by addition of Dowex 50 × 8 (H^+-form) and applied onto a column of the same ion-exchanger (100 ml). After washing with water to drop of UV absorption and conductivity of the eluate to the original values, the product was eluted with 2·5% ammonia, the UV-absorbing eluate was taken down in vacuo and the residue was codistilled with ethanol (2 × 50 ml). The brownish material was mixed with ethanol,

filtered, washed with ether and dried, yield 1·3 g (92%) of amorphous product, not melting up to 300°C. R_F 0·52 (S4). For $C_8H_{12}N_6O_2$ (224·2) calculated: 42·85% C, 5·40% H, 37·49% N; found: 42·61 C, 5·58% H, 37·22% N. UV (pH 7): 267.

9-(*RS*)-(2,3-Dihydroxypropyl)-6-hydroxylaminopurine (*III*)

A mixture of hydroxylamine hydrochloride (3·5 g, 50 mmol) and 1M methanolic sodium methoxide (50 ml) was stirred at room temperature overnight. Compound *XIII* (2·6 g, 8·3 mmol) was added and the mixture was heated in an autoclave to 120°C for 7 h. The further work-up was carried out in the same manner as described for compound *IIk* and afforded 1·4 g (75%) of chromatographically homogeneous compound *III*, not melting up to 260°C. For $C_8H_{11}N_5O_3$ (225·2) calculated: 42·66% C, 4·92% H, 31·10% N; found: 42·80% C, 5·12% H, 30·78% N. R_F 0·12 (S3), 0·54 (S4). UV (pH 2, 7, 12): 263nm (pH-independent).

9-(*RS*)-(2,3-Dihydroxypropyl)-6-thiopurine (*IIm*)

A mixture of compound *XIII* (6·3 g, 20 mmol), thiourea (2 g, 26·5 mmol) and anhydrous ethanol (100 ml) was refluxed with stirring and exclusion of moisture for 1 h. The originally clear solution deposited the product. The mixture was cooled with ice, the product was filtered, washed with ethanol and dissolved in 200 ml 0·1 mol l^{-1} sodium methoxide in methanol. After standing for 16 h, the mixture was neutralized with Dowex 50, filtered, evaporated in vacuo and the residue crystallized from water. Yield 3·0 g (66%) of compound *IIm*, chromatographically homogeneous and identical with an authentic material[11]. For $C_8H_{10}N_4O_2S$ (226·3) calculated: 42·46% C, 4·45% H, 24·77% N, 14·17% S; found: 42·47% C, 4·37% H, 25·17% N, 14·2°% S. R_F 0·27 (S3), 0·48 (S4). UV (H_2O): 325 nm.

9-(*RS*)-(2,3-Dihydroxypropyl)-2-methyladenine (*IIc*)

A mixture of 2-methyladenine (1·5 g, 10 mmol), potassium carbonate (3 g), dimethylformamide (25 ml) and compound *IIIb* (2 ml) was refluxed under stirring and exclusion of moisture for 10 h. The mixture was filtered while hot, the solid was washed with dimethylformamide, the filtrate was taken down at 50°C/13 Pa and the residue was extracted with boiling chloroform (300 ml). The chloroform was evaporated and the residue was purified by chromatography on two plates of silica gel in the system S2. The product zones were eluted with methanol (500 ml), the solvent was evaporated and the product was crystallized from ethyl acetate–light petroleum; yield 1·82 g (69%) of compound *VIIb*, m.p. 173–174°C. For $C_{12}H_{17}N_5O_2$ (263·3) calculated: 54·73% C, 6·51% H, 26·60% N; found: 54·54% C, 6·07% H, 26·30% N. MS: 263 (M^+), 248 (M − CH_3), 205 (M − $(CH_3)_2CO$), 188 (M − OH), 150 (BH_2), 149 (BH). R_F 0·40 (S2).

A solution of compound *VIIb* (1·75 g, 6·6 mmol) in 0·25M sulfuric acid (50 ml) was set aside overnight, diluted with water (100 ml), neutralized with saturated barium hydroxide solution and briefly boiled. The suspension was filtered through Celite which was then washed with boiling water (500 ml) and the filtrate was evaporated in vacuo. The residue was dissolved in 80% ethanol (100 ml), mixed with ether (200 ml) and the mixture was cooled in a refrigerator overnight. The crystallized product *IIc* (1·30 g, 87%) was collected; m.p. 258°C. For $C_9H_{13}N_5O_2$ (223·2) calculated: 48·42% C, 5·87% H, 31·38% N; found: 48·25% C, 5·56% H, 31·34% N. R_F 0·34 (S3), 0·68 (S4). UV (pH 1, 7, 12): 263 (14 500), no change with pH. ^{1}H NMR $(CD_3)_2SO$): 2·40 s, 3 H (CH_3); 3·34 m, 2 H (3′-CH_2); 3·86 m, 2 H (2′-CH); 3·98 dd + 4·27 dd, 2 H (1′-CH_2, $J(1',2') = 3·0$, $J(1'',2') = 7·5$, $J(\text{gem}) = -13·0$); 4·97 t, 1 H (3′-OH, $J(\text{OH},3'\text{-H}) = 6·0$);

5·11 d, 1 H (2′-OH), J(OH,2′-H) = 5·4); 7·06 br s, 2 H (6-NH_2); 7·95 s, 1 H (8-H); + CD_3COOD: 3·38 2 × dd, 2 H (3′-CH_2, J(3′,2′) = 5·0, J(3″,2′) = 6·0, J(gem) = −11·0).

7-(*RS*)-(2,3-Dihydroxypropyl)-4-aminopyrazolo[3,4-*d*]-pyrimidine (*IIq*)

A mixture of 4-aminopyrazolo[3,4-*d*]pyrimidine (6.7 g, 50 mmol), potassium carbonate (14·5 g, 105 mmol) and dimethylformamide (100 ml) was preheated to 140°C under reflux condenser equipped with calcium chloride tube. Chloro derivative *IIIb* (ref.[11], 15 g, 0·1 mol) was added under stirring at 140°C and the stirring was continued for 8 h. Then, a second portion of compound *IIIb* (7·5 g, 50 mmol) was added and the heating was continued for further 6 h. The hot mixture was filtered, the solid was washed with dimethylformamide (50 ml) and the filtrate was taken down at 50°C/13 Pa. The residue was extracted with chloroform (500 ml), the solvent was evaporated and the residue was chromatographed on a column of silica gel (300 ml).The product was eluted with chloroform–methanol (19 : 1) and crystallized from ethyl acetate–light petroleum; yield 6·7 g (54%) of compound *VIII*, m.p. 135—136°C. For $C_{11}H_{15}N_5O_2$ (249·3) calculated: 53·00% C, 6·07% H, 28·10% N; found: 52·67% C, 6·02% H, 27·80% N. ^{1}H NMR ($(CD_3)_2SO$): 1·22 + 1·28 2 × bs, 6 H (isopropylidene, J = 0·6); 4·22—4·54 m, 3 H (1′-CH_2 + 2′-CH); 3·86 dd + 3·99 dd, 2 H (3′-CH_2, J(3′,2′) = 6·2, J(3″,2′) = 5·2, J(gem) = 8·6); 7·66 br s, 2 H (4-NH_2); 8·09 s, 1 H (2-H); 8·17 s, 1 H (5-H); ^{13}C NMR: 25·40 + 26·91, 2 × s (isopropylidene-CH_3); 49·11 s, 1′-CH_2; 66·74 s (3′-CH_2); 73·95 s (2′-H); 100·11 s (5-C); 132·36 s (9-C); 153·64 s (6-C); 156·10 s (2-C); 158·22 s (4-C).

A solution of compound *VIII* (6·2 g, 25 mmol) in 0·25M sulfuric acid (200 ml) was set aside overnight and worked up as described for compound (*S*)-*IIt* (hydrolysis of *VIa*). Crystallization from 80% ethanol (ether added to turbidity) furnished 4·1 g (78%) of compound *IIq*, m.p. 178—189°C. For $C_8H_{11}N_5O_2$ (209·2) calculated: 45·92% C, 5·30% H, 33·48% N; found: 45·72% C, 5·34% H, 33·35% N. ^{1}H NMR ($(CD_3)_2SO$): 3·39 m, 2 H (3′-CH_2); 4·01 m, 1 H (2′-CH); 4·29 d, 2 H (1′-CH_2, J(1′,2′) = 6·2); 4·74 t, 1 H (3′-OH, J(OH, 3′-H) = 5·3); 7·65 br s, 2 H (4-NH_2); 8·09 s, 1 H (2-H); 8·17 s, 1 H (9-H). ^{13}C NMR: 50·20 s (1′-CH_2); 64·04 s (3′-CH_2); 70·55 s (2′-C); 100·32 s (5-C); 132·04 s (9-C); 153·67 (6-C, J(1′,6) = 0); 155·93 s (2-C); 158·33 s (4-C).

1-(*RS*)-Dihydroxypropyl-5-fluorouracil (*IIv*)

A mixture of 5-fluorouracil (*XIV*, 8·2 g, 63 mmol), hexamethyldisilazane (60 ml) and ammonium sulfate (0·6 g) was stirred and refluxed (140°C) for 8 h (calcium chloride tube). After evaporation at 2 kPa (bath temperature up to 80°C), the residue was dissolved in acetonitrile (60 ml). Allyl bromide (60 ml) was added and the mixture was refluxed for 5 h under exclusion of moisture. The solvent was evaporated in vacuo and the residue was codistilled with ethanol, dissolved in chloroform (100 ml), filtered through Celite and the filtrate was chromatographed on a column of silica gel (300 ml) in chloroform. Elution with chloroform–methanol (49 : 1) and crystallization from ethanol–light petroleum afforded 9 g (84%) of 1-allyl-5-fluorouracil (*XVI*), R_F 0·46 (S2). This product was dissolved in 50% aqueous ethanol (140 ml), and sodium chlorate (15 g, 250 mmol), followed by osmium tetroxide (15 mg), was added. The mixture was stirred under reflux condenser at 50°C for 5 h and evaporated in vacuo. The residue was mixed with ethanol (200 ml), filtered, washed with ethanol (100 ml; the solid contained only inorganic salts), and the filtrate was taken down in vacuo. The residue was codistilled with toluene (2 × 50 ml), mixed with acetone (130 ml) and 2,2-dimethoxypropane (70 ml), and 5M-HCl in dimethylformamide was added to acid reaction. The mixture was stirred overnight, neutralized with triethylamine, evaporated in vacuo, and the residue was dissolved in water (100 ml) and extracted with chloro-

form (5 × 50 ml). After drying and evaporation of the chloroform in vacuo, the residue (R_F 0·40, S2) was set aside overnight with 0·25M sulfuric acid (100 ml) and neutralized with saturated sodium hydroxide solution. The mixture was briefly boiled, filtered through Celite and washed with hot water (500 ml). The filtrate was evaporated in vacuo, the residue was dissolved in water (20 ml) and applied onto a column of octadecyl silica gel (30 μm, 200 ml) in water. The column was washed with water and the UV-absorbing fraction was evaporated in vacuo. The residue was codistilled with ethanol (2 × 50 ml) and crystallized from ethanol (ether added to turbidity) to afford 6·2 g (57%) of compound *IIv*, m.p. 139–141°C. For $C_7H_9FN_2O_4$ (204·2) calculated: 41·18% C, 4·44% H, 9·31% F, 13·72% N; found: 40·98% C, 4·72% H, 9·47% F, 13·11% N.

Preparation of Racemic Mixture of Isomeric Phosphonylmethyl Derivatives *XX* and *XXI* — General Procedure

Method A. Compound *XVII* (0·40 ml, 3·9 mmol) was added to a stirred suspension of compound *II* (3 mmol) in triethyl phosphate (10 ml). The mixture was stirred in a stoppered flask at room temperature overnight, and poured into ether (200 ml). The precipitate was filtered, washed with ether (100 ml) and dried in vacuo under exclusion of moisture (about 15 min). (A sample (about 10 mg) of the mixture was dissolved in 0·2 ml of 0·3M buffer S5 and analyzed by HPLC. (The composition of the mixture of *XVIII* and *XIX* is given in Table I.) Water (20 ml) was added, the mixture was refluxed for 12 h and then neutralized with 1M sodium hydroxide. After evaporation in vacuo, the residue was heated to 80°C with 2M sodium hydroxide (25 ml) for 6 h. Dowex 50 × 8 (H^+-form) was added to acid reaction and the mixture was poured on a column of the same ion-exchanger (100 ml). After elution with water to drop of UV absorption and conductivity of the eluate to the original values, the product was eluted with 2·5% aqueous ammonia and the UV-absorbing eluate was evaporated in vacuo. The residue was applied onto a column of Sephadex A-25 (HCO_3^- form, 100 ml). The column was washed with water to drop of UV absorption and then the material was eluted with a linear gradient of 0–0·2M buffer S5 (à 1 l). The UV-absorbing product fraction was collected, evaporated, the residue was codistilled with methanol (3 × 50 ml), dissolved in water (20 ml) and applied onto a column of Dowex 50 × 8 (Na^+-form, 50 ml). Elution with water afforded a UV-absorbing eluate which on evaporation in vacuo, codistillation with ethanol (2 × 20 ml) and precipitation from methanol (5 ml) with ether (200 ml) gave disodium salt of mixture of racemic isomers *XX* and *XXI* (the content of (*RS*)-*XXI* corresponds to that of the isomer (*RS*)-*XIX* given for t_∞ in Table I). The yields and characteristics of thus-prepared compounds are given in Table II. This method was applied to all the compounds *II*, except *IIf* and *IIv*. Under the described conditions, compounds *IIt* and *IIu* were converted quantitatively into compounds *XXr*, *XXIr* and *XXs* (vide infra).

Method B. The reaction with compound *IIv* was performed in the same manner as described for Method *A*. After the end of the reaction, the mixture was mixed with ether (100 ml) and light petroleum (100 ml), the separated precipitate was filtered, washed with light petroleum–ether (1 : 1) and dried in vacuo. The subsequent work-up procedure was executed according to Method *A*. The combined filtrates were extracted with 0·4M buffer S5 (50 ml) and water (2 × 50 ml), the extract was taken down in vacuo and the residue was codistilled with methanol (2 × 50 ml) and isomerized in 1M hydrochloric acid (25 ml) for 8 h at 80°C. For the further processing according to Method *A* both the portions were combined.

Method C (with isolation of the intermediate). Compound *XVII* (1·40 ml, 13·7 mmol) was added to a suspension of compound (*RS*)-*IId* (1·55 g, 10 mmol) in triethyl phosphate (35 ml) and the mixture was stirred in a stoppered flask overnight. Ether (200 ml) was added, the precipitate was filtered, washed with ether and shortly dried. The material was refluxed with water

(70 ml) for 20 h. After cooling, the crystallized product was filtered, washed with ice-cold water, ethanol, ether and dried in vacuo, yielding 2·4 g (72%) of a mixture of compounds *XVIIId* and *XIXd*. For $C_{10}H_{15}ClN_5O_3PS$ (351·8) calculated: 34·14% C, 4·30% H, 10·08% Cl, 19·91% N, 8·82% P; found 34·41% C, 4·34% H, 10·32% Cl, 19·68% N, 8·66% P.

This product (1·92 g, 6 mmol) was heated with 2M sodium hydroxide to 80°C for 8 h and worked up as described for Method *A*. Sodium salts of (*RS*)-*XXd* and *XXId*, identical with the material obtained by Method *A*, were obtained in 76% yield based on the mixture of *XVIIId* and *XIXd*.

The described procedure was also used for the preparation of chloromethylphosphonyl derivatives (*RS*)-*XVIIIc* and *XIXc* in a yield of 80% from 3 mmol of compound (*RS*)-*IIc*: for $C_{10}H_{15}ClN_5O_3P.2H_2O$ (355·8) calculated: 33·75% C, 5·38% H, 9·96% Cl, 19·69% N, 8·72% P; found: 33·98% C, 5·22% H, 9·73% Cl, 29·94% N, 8·67% P.

Preparation of a Mixture of Racemic Phosphonylmethoxy Derivatives *XXf* and *XXIf* by Deamination of *XXe* and *XXIe*

3-Methylbutyl nitrite (2 ml) was added to a solution of hydrate of disodium salt of *XXe* and *XXIe* (for isomeric composition see Table II; 0·77 g, 2 mmol) in 80% acetic acid (20 ml) and the mixture was allowed to stand overnight at room temperature. After evaporation in vacuo and codistillation with water (3 × 10 ml), the residue was dissolved in water (20 ml), the solution adjusted to pH 9 with ammonia and chromatographed on a column of Sephadex A-25 according to Method *A*. Yield 72% of sodium salt of mixture of compounds *XXf* and *XXIf* (for characteristics see Table II).

9-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)hypoxanthine ((*S*)-*XXIj*) (HPMPHx)

3-Methylbutyl nitrite (2 ml) was added to a solution of dihydrate of disodium salt of HPMPA (*I*, 0·76 g, 2 mmol) in 80% acetic acid (20 ml) and the mixture was set aside in a stoppered flask overnight. After evaporation in vacuo, the residue was codistilled with water (3 × 10 ml), dissolved in water (10 ml) and applied onto a column of Dowex 50 × 8 (H^+-form, 50 ml). The column was eluted with water and the UV-absorbing eluate (considerable retention) was taken down in vacuo. The residue was codistilled with ethanol (2 × 20 ml) and crystallized from ethanol–ether, affording compound (*S*)-*XXIj* (67%) of the same parameters as the racemic mixture (Table II). For $C_9H_{13}N_4O_6P$ (304·3) calculated: 35·52% C, 4·30% H, 18·42% N, 10·20% P; found: 35·74% C, 4·22% H, 18·66% N, 10·31% P.

9-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)-2,6-diaminopurine ((*S*)-*XXIe*) (HPMPDAP)

A mixture of compound (*S*)-*IIe* (6·72 g, 30 mmol, dried by codistillation with pyridine (3 × × 100 ml), triturated with ether, filtered and dried at 50°C/13 Pa over phosphorus pentoxide), triethyl phosphate (150 ml) and compound *XVII* (4·2 ml, 6·88 g, 41 mmol) was stirred in a stoppered flask at room temperature overnight and poured into ether (600 ml). The product was filtered, washed with ether, dried in vacuo for 30 min under exclusion of moisture and refluxed with water (180 ml) for 14 h. After cooling to room temperature, the crystalline product was filtered, washed with water, acetone, ether and dried in vacuo to give 2·0 g (21%) of hydrate of compound *XIXe*, homogeneous according to HPLC. For $C_9H_{14}ClN_6O_4.H_2O$ (323·7) calculated: 33·39% C, 4·98% H, 10·95% Cl, 25·97% N; found: 33·18% C, 5·12% H, 11·09% Cl, 26·12% N. For values of k and E_{Up} see Table I.

The aqueous filtrate was concentrated in vacuo to about 100 ml and left to crystallize in a refrigerator. An analogous work-up procedure gave 4·5 g (46%) of compound *XIXe*, homogeneous according to HPLC. For $C_9H_{14}ClN_6O_4.H_2O$ (323·7) calculated: 33·39% C, 4·98% H, 10·95% Cl, 25·97% N; found: 33·26% C, 5·03% H, 10·56% Cl, 26·14% N.

The residue after crystallization of compound *XIXe* was deionized on a column of Dowex 50 × 8 (H^+-form, 200 ml), the ammonia eluate was evaporated and the residue, containing a mixture of both isomers (about 85% of compound *XIXe*), was purified by preparative liquid chromatography on a reversed phase in 0·05M buffer S5. The obtained pure fraction of (*S*)-*XIXe* (triethylammonium salt) was combined with the free acid form of the same compound (vide supra) and heated with 2M sodium hydroxide (150 ml) to 80°C for 12 h. The mixture was neutralized with Dowex 50 × 8 (H^+-form), poured on a column of the same ion-exchanger (200 ml) and washed with water to drop of UV absorption of the eluate to the original value. Elution with 2·5% ammonia gave a UV-absorbing eluate which was evaporated in vacuo and the residue was converted into the sodium salt on a column of Dowex 50 × 8 (Na^+ form, 150 ml). After codistillation with ethanol, the sodium salt was obtained by precipitation with ether from methanol; yield 5·0 g (44%) of hydrate of disodium salt of compound (*S*)-*XXIe* (HPLC pure). For $C_9H_{13}N_6O_5PNa_2.H_2O$ (380·3) calculated: 22·10% N, 8·16% P; found: 21·78% N, 7·96% P. $[\alpha]_D^{20}$ −40·1° (*c* 0·5, 1M-HCl).

In the similar manner, compound (*S*)-*XVIII* (vide supra) was converted into the 3′-isomer (*S*)-*XXe* (reaction in 50 ml of 2M sodium hydroxide). Yield 1·7 g (15%) of hydrate of disodium salt of compound (*S*)-*XX*; its chromatographic and electrophoretic properties were the same as those of the 2′-isomer.

9-((*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)-3-deazaadenine ((*S*)-*XXIp*)

Compound *XVII* (0·4 ml) was added under stirring to a solution of compound (*S*)-*IIp* (0·48 g, 2·3 mmol) in triethyl phosphate (9 ml) and the stirring was continued overnight at room temperature. The mixture was diluted with ether (80 ml), filtered, the solid was washed with ether, dried in vacuo and then refluxed with water (14 ml) for 10 h. The mixture was made alkaline with ammonia (to pH 8) and applied onto a column of Amberlite IRC 50 (H^+-form, 200–400 mesh, 350 ml). The column was washed with water and the UV-absorbing fractions (20 ml each) were analyzed by HPLC (see Table I). Fractions containing the pure 3′-isomer *XIXp* were combined, neutralized with ammonia and evaporated in vacuo. The obtained product (0·3 g, 1 mmol) was heated with 2M sodium hydroxide (20 ml) to 80°C for 6 h and the mixture was deionized on a column of Dowex 50 × 8 (H^+-form, 200 ml). The ammonia eluate was taken down and purified by chromatography on a column of Dowex 1 × 2 (acetate). After washing with water to drop of UV absorption to the original value, the product was eluted with 0·5M acetic acid. The eluent was evaporated in vacuo, the residue was codistilled with water (3 × 20 ml) and ethanol and the product was obtained by crystallization from a concentrated aqueous solution after addition of 4 volumes of ethanol and then ether to turbidity. Yield 0·25 g (71%) of compound (*S*)-*XXIp*, not melting up to 250°C. For analytical and other data see Table II. ^{1}H NMR (D_2O + NaOD): 3·45 dd, 1 H (O–CH_2, J(3′,2′) = 5·4, J(gem) = 12·0); 3·50 d, 2 H (P–CH_2, J(P–CH) = 8·9); 3·69 dd, 1 H (O–CH_2, J(3′,2′) = 3·6, J(gem) = 12·0); 3·63 br pent, 1 H (O–CH); 4·36 2 × dd, 2 H (N–CH_2, J(1′,2′) = 5·0, J(1″,2′) = 6·0, J(gem) = 15·0); 7·00 d, 1 H (3-H arom., J(2,3) = 6·0); 7·75 d (2-H arom., J(2,3) = 6·0); 8·20 s, 1 H (8-H arom.). UV (pH 2): 262 (11 600); (pH 12); 264 (11 900). $[\alpha]_D^{20}$ −19·8° (*c* 0·5, 0·1M-HCl).

1-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine ((*S*)-*XXIt*) and -uracil ((*S*)-*XXIr*), and 1-(*S*)-(2-Hydroxy-3-phosphonylmethoxypropyl)cytosine ((*S*)-*XXt*) and -uracil ((*S*)-*XXr*)

Compound *XVII* (1·2 ml) was added to a solution of compound (*S*)-*IIt* (9 mmol, dried as described for compound (*S*)-*IIe*) in triethyl phosphate (30 ml). The mixture was stirred at room temperature overnight, mixed with ether (300 ml), filtered, the solid was washed with ether and dried in vacuo for 15 min under exclusion of moisture. This product was dissolved in water (60 ml) and allowed to stand at room temperature for 2 days (under these conditions, the original content of the 3′-isomer (42%) did not change, the conversion of compound *IIt* was quantitative). The solution was deionized on a column of Dowex 50 × 8 (H^+-form, 200 ml) and eluted with water. The fractions (20 ml) were analyzed by HPLC and those enriched in the individual isomers were combined and evaporated in vacuo. The resulting products were codistilled with ethanol, precipitated with ether from methanol (10 ml), collected on filter and dried in vacuo, yielding 1·50 g (56%) of 1st fraction containing 85% of isomer (*S*)-*XVIIIt* and 0·70 g (26%) of 2nd fraction with 90% of isomer (*S*)-*XIXt*. The 1st fraction was subjected to preparative reversed-phase HPLC in 0·05M buffer S5 (100 mg injections) and fractions of the pure isomers were taken down. The pure isomer (*S*)-*XVIIIt* was heated with 2M sodium hydroxide (50 ml) to 70°C for 6 h, the mixture was neutralized with Dowex 50 × 8 (H^+-form) and the suspension was poured on a column of the same ion-exchanger (100 ml). The material was eluted with water and the UV-absorbing eluate was neutralized with ammonia and taken down. The residue was dissolved in water (20 ml), applied onto a column of Dowex 50 × 8 (Li^+-form, 100 ml), eluted with water and the UV-absorbing eluate was evaporated in vacuo. The residue was stirred with ethanol (50 ml) for 30 min, mixed with acetone (50 ml), filtered and the solid was washed with acetone–ethanol (1 : 1), acetone, and ether, and dried. Yield 1·10 g (37% based on (*S*)-*IIt*) of hydrate of dilithium salt of compound (*S*)-*XXr*. For $C_8H_{11}N_2O_7PLi_2.2H_2O$ (328·2) calculated: 8·54% N, 9·46% P; found: 8·70% N, 9·32% P. Properties of the product are the same as given in Table II. UV (pH 2): 260 nm.

The column of Dowex 50 × 8 (H^+-form) from the above-mentioned chromatography was eluted with 2·5% ammonia. Evaporation in vacuo and conversion into the sodium salt (Method *A*) afforded 500 mg (17% based on (*S*)-*IIt*) of disodium salt of compound (*S*)-*XXt*. For $C_8H_{12}N_3O_6PNa_2$ (323·2) calculated: 13·00% N, 9·60% P; found: 12·94% N, 9·42% P. UV (pH 2): 280 nm.

The second fraction (enriched with compound *XIXt*), combined with the pure compound *XIXt* obtained by preparative HPLC of the first fraction, was chromatographed on a column of Dowex 50 × 8 (H^+-form, 100 ml) in water and the fractions (20 ml) were analyzed by HPLC. Fractions containing pure compound were combined, taken down in vacuo, mixed with ethanol and filtered to give 0·12 g (4·5% based on (*S*)-*IIt*) of (*S*)-*XVIIIt* (pure according to HPLC) which did not melt up to 260°C. For $C_8H_{13}ClN_3O_5P$ (297·7) calculated: 32·27% C, 4·40% H, 11·91% Cl, 14·11% N, 10·43% P; found: 32·40% C, 4·45% H; 12·23% Cl, 13·95% N; 10·70% P. Further elution and analogous work-up afforded 0·70 g of HPLC-pure isomer (*S*)-*XIXt* which was treated with sodium hydroxide similarly as described above for compound (*S*)-*XVIIIt*. Deionization gave 0·30 g (10% based on compound (*S*)-*IIt*) of dilithium salt of compound (*S*)-*XXIr*; UV (pH 2): 260 nm. The ammonia eluate on deionization furnished 0·50 g (18% based on compound (*S*)-*IIt*) of disodium salt of compound (*S*)-*XXIt*. For $C_8H_{12}N_3Na_2O_6P$ (323·2) calculated: 13·00% N, 9·60% P; found: 13·24% N, 9·67% P. ^{13}C NMR (D_2O): 50·06 s, (1′-CH_2); 60·69 s (3′-CH_2); 67·54 d (P-CH_2, ^1J(P,C) = 153·5); 80·18 d (2′-CH_2, ^3J(P,C) = 11·5); 95·61 s (5-C); 148·01 s (6-C); 158·38 s (2-C); 166·44 s (4-C). UV (pH 2): 280 nm.

1-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)-5-methyl-cytosine ((*S*)-*XXIu*) and -thymine ((*S*)-*XXIs*), 1-(*S*)-(2-Hydroxy-3-phosphonylmethoxypropyl)-5-methylcytosine ((*S*)-*XXu*) and -thymine ((*S*)-*XXs*)

The reaction was performed with compound (*S*)-*IIu* (2 g, 10 mmol) and chloride *XVII* (1·4 ml) in triethyl phosphate (40 ml) as described for compound (*S*)-*XXIt* (and other compounds). After precipitation of the intermediate with ether and drying, the product was refluxed in water (60 ml) for 12 h and chromatographed on a column of Dowex 50 × 8 (H^+-form, 250 ml) in water. The fractions (20 ml) were analyzed by HPLC, the corresponding fractions were combined, taken down and the enriched fractions were rechromatographed on columns (150 ml) of the same ion exchanger under the same conditions. Evaporation in vacuo, mixing with ethanol and filtration afforded 2·0 g (61%) of hydrate of compound *XIXu*, not melting up to 260°C. For $C_9H_{15}ClN_3O_5P.H_2O$ (329·8) calculated: 32·78% C, 5·20% H, 10·75% Cl, 12·74% N, 9·41% P; found: 32·50% C, 5·58% H, 10·62% Cl, 12·54% N, 9·11% P. Further fractions gave 1·1 g (32%) of pure (HPLC) isomer *XVIIIu* of properties similar to those of the 3′-isomer *XIXu* (Table I).

Compound (*S*)-*XIXu* (5 mmol) was heated with 2M sodium hydroxide (40 ml) to 80°C for 12 h and processed as described above for the reaction of the cytosine derivative ((*S*)-*IIt*). Yield 1·1 g (68%) of hydrate of dilithium salt of compound (*S*)-*XXIs*. For $C_9H_{13}.N_2O_7PLi_2.H_2O$ (324·2) calculated: 8·64% N, 9·57% P; found: 8·22% N, 10·11% P. UV (pH 2): 266 nm. The ammonia eluate afforded dihydrate of disodium salt of compound (*S*)-*XXIu* (0·50 g, 27%). For $C_9H_{14}N_3O_6PNa_2.2H_2O$ (373·3) calculated: 11·26% N, 8·31% P; found: 10·92% N, 8·17% P. UV (pH 2): 287 nm.

Compound (*S*)-*XVIIIu* (3 mmol) was heated with sodium hydroxide in a similar manner. The above-described work-up of the reaction mixture gave hydrate of dilithium salt of compound (*S*)-*XXIs* (λ_{max} 266 nm (pH 2)) (0·60 g, 61%) and dihydrate of disodium salt of compound (*S*)-*XXIu* (λ_{max} 287 nm (pH 2)) (0·22 g, 20%). Characteristics of these compounds were identical with those of the racemic compounds (Table II).

9-(*S*)-(2-Benzoyloxy-3-hydroxypropyl)-N^2-benzoylguanine ((*S*)-*XXIV*)

Compound (*S*)-*IIn* (4·82 g, 21·4 mmol) was codistilled with pyridine and suspended in pyridine (120 ml). Chlorotrimethylsilane (18 ml, 14·6 g, 134 mmol) was added and the mixture was stirred at room temperature for 1 h. After addition of benzoyl chloride (14 ml, 16·9 g, 120 mmol), the stirring at room temperature was continued for 2 h, the mixture was cooled with ice and decomposed by dropwise addition of ice-cold water (23 ml) with stirring. After 5 min concentrated aqueous ammonia (50 ml) was added during 10 min under stirring. The mixture was stirred for 30 min in an ice bath and evapoiared. The residue was shaken with water (200 ml) and chloroform (100 ml), filtered and the solid was washed with chloroform, acetone, ether and dried; 6·1 g (87%) of compound (*S*)-*XXII*, m.p. 228–229°C. For $C_{15}H_{15}N_5O_4$ (329·3) calculated: 54·70% C, 4·59% H, 21·27% N; found: 54·79% C, 4·50% H, 21·18% N. ^{1}H NMR ($(CD_3)_2SO$): 3·40 2 × dt, 2 H (3′-CH_2); 3·86 m, 1 H (2′-CH); 3·99 dd + 4·27 dd (1′-CH_2, $J(1',2') = 2·5$, $J(1'',2') = 8·5$, $J(gem) = 13·4$); 4·82 t, 1 H (3′-OH, $J(3',OH) = 5·2$); 5·12 d, 1 H (2′-OH, $J(2',OH) = 5·0$); 7·43–7·83 m, 3 H + 7·95–8·18 m, 2 H (arom); 7·95 s, 1 H (8-H); 11·95 br s, 1 H + 12·25 br s, 1 H (2 × NH). R_F 0·18 (S3), $[\alpha]_D^{20}$ −51·1° (*c* 0·5, dimethylformamide).

This compound (5·8 g, 17·5 mmol) was codistilled with pyridine (2 × 100 ml), suspended in pyridine (175 ml) and mixed with 4-dimethylaminopyridine (0·3 g) and dimethoxytrityl chloride (6·8 g, 20 mmol). The mixture was stirred in a stoppered flask at room temperature overnight, methanol (4 ml) was added and, after 1 h, the mixture was poured into saturated solution of sodium hydrogen carbonate (700 ml) and extracted with chloroform (3 × 100 ml). The extract

was washed with water (2 × 50 ml), the solvent was evaporated in vacuo and the residue was codistilled with toluene (3 × 100 ml). The product was dissolved in hot toluene and ether (2 parts) was added. The arising gel crystallized overnight in a refrigerator, the product was collected, washed with ether and dried in vacuo. Yield 10·4 g (95%) of chromatographically pure (*S*)-*XXIIIa*, m.p. 136°C. For $C_{36}H_{33}N_5O_6$ (631·7) calculated: 68.44% C, 5·26% H, 11·07% N; found: 69·07% C, 5·46% H, 11·32% N. R_F 0·28 (S2), $[\alpha]_D^{13}$ −13·4° (*c* 0·5, dimethylformamide).

Triethylamine (3 ml) was added to a mixture of compound (*S*)-*XXIIIa* (9·5 g, 15 mmol), benzoyl cyanide (2·9 g, 22 mmol) and chloroform (100 ml) and the mixture was stirred under exclusion of moisture for 2 h. Methanol (2 ml) was added, the mixture was evaporated in vacuo and the remaining compound (*S*)-*XXIIIb* (R_F 0·62 in S1, 0·80 in S2) was dissolved in a mixture of chloroform (110 ml), methanol (100 ml) and water (10 ml). After addition of trifluoroacetic acid (2·5 ml), the mixture was set aside at room temperature and the reaction was monitored by TLC in S2. After 1 h the reaction was quantitative, triethylamine (4·5 ml) was added and the mixture was taken down in vacuo. The residue was codistilled with acetone, mixed with acetone (50 ml), and ether (200 ml) was added. After cooling with ice, the product was filtered, washed with ether and dried; yield 5·9 g (91%) of compound (*S*)-*XXIV*, m.p. 193—194°C. For $C_{22}H_{19}$·.N_5O_5 (433.4) calculated: 60·97% C, 4·42% H, 16·16% N; found: 60·54% C, 4·40% H, 16·11% N. R_F 0·18 (S1). $[\alpha]_D^{20}$ −90·1° (*c* 0·5, dimethylformamide).

9-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)guanine ((*S*)-*XXIn*) (HPMPG)

Compound *XVII* (2.3 ml, 22·4 mmol) was added to a suspension of compound (*S*)-*XXIV* (5·6 g, 13 mmol) in triethyl phosphate (40 ml), the mixture was stirred in a stoppered flask to homogeneity (30 min), set aside overnight and poured with stirring into ether (400 ml). The solid was filtered, washed with ether and dried in vacuo for 1 h. The product was heated to 80°C with 2M sodium hydroxide (120 ml) for 8 h. Dowex 50 × 8 (H^+-form) was added to a slightly acid reaction and the mixture was made alkaline with triethylamine. The ion exchanger was filtered, washed with 50% methanol (200 ml) and water (200 ml), the combined filtrates were concentrated in vacuo to about 50 ml and this solution was applied onto a column of Dowex 50 × 8 (H^+-form, 300 ml). The column was washed with 30% aqueous methanol to drop of UV absorption of the eluate (benzoic acid) to the original value and the product was eluted with 2·5% ammonia. The ammonia UV-absorbing eluate was evaporated in vacuo and the residue in water (50 ml) was applied onto a column of Dowex 50 × 8 (Na^+-form, 200 ml). Elution with water gave a UV-absorbing fraction which was taken down and the residue was codistilled with ethanol and precipitated from ethanol with ether. Yield 4·7 g (94%) of hydrate of disodium salt of compound (*S*)-*XXIn*. For $C_9H_{12}N_5O_6PNa_2.H_2O$ (381·3) calculated: 18·37% N, 8·14% P; found: 18·20% N, 8·00% P. UV (pH 2): 257 (11 400). $[\alpha]_D^{20}$ −39° (*c* 0·5, 0·1M-HCl). All other characteristics were identical with those of the racemate (Table II). ^{13}C NMR (D_2O): 43·44 d (1′-C); 60·82 s (3′-C); 68·38 d (P–C, ^1J(P,C) = 150·7); 80·56 d, (2′-C, ^3J(P,C) = 10·5); 117·42 (5-C); 139·33 (8-C);151·69 (4-C); 161·26 (2-C); 168·41 (6-C). According to HPLC (Table II), the compound is the homogeneous 2′-isomer.

1-(*S*)-(2,3-Dihydroxypropyl)-N^4-benzoylcytosine ((*S*)-*XXV*)

Method A. Compound (*S*)-*IIt* (27·8 g, 0·25 mol) was codistilled with pyridine (2 × 200 ml), suspended in pyridine (800 ml) and mixed with chlorotrimethylsilane (104 ml). The stirring was continued for 1 h under exclusion of moisture. Benzoyl chloride (94 ml) was added and the mixture was stirred at room temperature for 2 h. The mixture was cooled with ice and decomposed by dropwise addition of ice-cold water (162 ml) during 5 min. Concentrated ammonia (360 ml)

was added in the course of 10 min and after 30 min the mixture was evaporated in vacuo. The residue was crystallized from water, the mother liquor was taken down in vacuo, the residue was codistilled with ethanol (2 × 200 ml), mixed with ethanol (200 ml), filtered and the solid was washed with the same solvent (100 ml). The filtrate was evaporated with silica gel (100 g), codistilled with toluene (200 ml) and the silica gel was layered on a column of silica gel (300 ml) in chloroform. Elution with the system S1 afforded further portion of pure product (*S*)-*XXV*, m.p. 189–191°C; total yield 33·7 g (77%). For $C_{14}H_{15}N_3O_4$ (289·3) calculated: 58·12% C, 5·23% H, 14·53% N; found: 58·21% C, 4·98% H, 14·35% N. R_F 0·25 (S2). $[\alpha]_D^{20}$ −68·4° (*c* 0·5, dimethylformamide).

Method B. Benzoyl chloride (12 ml, 103 mmol) was added dropwise without cooling to a stirred solution of compound (*S*)-*VIa* (9 g, 40 mmol) in pyridine (80 ml) during 10 min. After stirring in a stoppered flask overnight, ethanol (20 ml) was added and after 20 min the mixture was evaporated in vacuo. The residue was triturated with a mixture of ethyl acetate (300 ml) and water (200 ml), filtered, washed with ethyl acetate, acetone and ether. The filtrate was stripped of organic solvents in vacuo, the aqueous solution was extracted with ethyl acetate (3 × 100 ml), the extract was dried over sodium sulfate, evaporated in vacuo and the residue was crystallized from ethyl acetate–light petroleum. After filtration and washing with ether, the material was dried and combined with the first crop; total yield 13·4 g (77%) of compound (*S*)-*XXVIII*, m.p. 187–188°C. For $C_{24}H_{23}N_3O_5$ (433·5) calculated: 66·51%C, 5·35% H, 9·70% N; found: 66·34% C, 5·36% H, 9·98% N. $[\alpha]_D^{20}$ −28·0° (*c* 0·5, dimethylformamide). R_F 0·60 (S1). ^{1}H NMR ($(CD_3)_2SO$): 1·25 + 1·35 2s, 6 H (isopropylidene); 3·62 dd, 1 H (J = 5·8; 8·5) + 4·00 m, 3 H + 4·33 m, 1 H (N–CH + O–CH); 6·64 d, 1 H (5-H); 8·14 d, 1 H (6-H); 7·25–8·0 m, 10 H (arom.).

This product (13 g, 30 mmol) was mixed with dioxane (300 ml) and conc. aqueous ammonia (50 ml) and stirred for 30 min. The solvent was evaporated and the residue was codistilled with dioxane (100 ml), mixed with light petroleum–ether (1 : 1), filtered and the collected product was washed with the same mixture and dried in vacuo. Yield 9·5 g (96%) of compound (*S*)-*XXIX*, m.p. 192–193°C. For $C_{17}H_{19}N_3O_4$ (329·4) calculated: 61·99% C, 5·82% H, 12·76% N; found: 61·68% C, 5·77% H, 12·54% N. R_F 0·40 (S1), $[\alpha]_D^{20}$ −52·4° (*c* 0·5, dimethylformamide); ^{1}H NMR ($(CD_3)_2SO$): 1·26 + 1·35 2 × s, 6 H (isopropylidene); 3·83 m, 1 H + 4·04 m, 3 H + 4·40 m, 1 H (N–CH + O–CH); 7·29 d, 1 H (5-H, J(5,6) = 7·0); 8·07 d, 1 H (6-H, J(5,6) = 7·0); 7·35–7·65 m, 3 H + 7·90–8·10 m, 2 H (arom.).

The obtained product (9 g, 27·3 mmol) was mixed with dioxane (50 ml) and 0·5M sulfuric acid (50 ml), the mixture was stirred to dissolution and set aside overnight. After dilution with water (200 ml), the mixture was neutralized with saturated solution of barium hydroxide, briefly boiled, filtered through Celite which was then washed with water (200 ml) and hot methanol (1 l). The filtrate was taken down in vacuo, the residue was codistilled with ethanol (2 × 100 ml) and crystallized from ethanol–ether; yield 6·0 g (76%) of compound (*S*)-*XXV*, identical with the product prepared by method *A*. ^{1}H NMR ($(CD_3)_2SO$): 3·32 m, 2 H (3′-CH_2); 3·77 m, 1 H (2′-CH); 3·40 dd, 1 H + 4·16 dd, 1 H (1′-CH_2, J(1′,2′) = 2·5, J(1″,2′) = 4·0, J(gem) = −12·5); 4·72 t, 1 H (3′-OH, J(3′, OH) = 5·3); 5·02 d, 1 H (2′-OH, J(2′, OH) = 5·3); 7·27 d, 1 H (5-H, J(5, 6) = 7·0); 8·02 d, 1 H (6-H); 11·11 br s, 1 H (NH); 7·35–7·70 m, 3 H + 7·90–8·10 m, 2 H (arom.).

1-(*S*)-(2-Benzoyloxy-3-hydroxypropyl)-N^4-benzoylcytosine ((*S*)-*XXVII*)

Compound (*S*)-*XXV* (33·5 g, 126 mmol) was dried by codistillation with pyridine (2 × 150 ml),

suspended in pyridine (1 l), mixed with dimethoxytrityl chloride (45 g, 132 mmol) and 4-dimethylaminopyridine (2.5 g), stirred to dissolution and set aside overnight. Methanol (20 ml) was added and, after standing for 1 h, the mixture was poured into a saturated solution of sodium hydrogen carbonate (3 l). The mixture was extracted with chloroform (4 × 500 ml), the combined extracts were washed with water (3 × 200 ml), taken down in vacuo, the residue was codistilled with toluene (3 × 100 ml) and dissolved in benzene (250 ml). The obtained solution was added dropwise during 30 min into stirred light petroleum (4 l). The product was collected, washed with light petroleum and dried in vacuo. The chromatographically pure compound *(S)-XXVIa* (R_F 0·40, S1) was obtained in quantitative yield.

This product was dissolved in chloroform (500 ml), mixed with benzoyl cyanide (21·7 g, 0·17 mol) and triethylamine (15 ml) and the mixture was allowed to stand at room temperature overnight. Methanol (10 ml) was added, the mixture was taken down in vacuo and the residue was chromatographed on a column of silica gel (1 400 ml) in chloroform, containing 0·5% of triethylamine, affording 65·5 g (81%) of amorphous *(S)-XXVIb*; R_F 0·65 (S1).

The obtained product (65 g, 93·5 mmol) was dissolved in a mixture of chloroform (500 ml), methanol (430 ml) and water (43 ml), and trifluoroacetic acid (11 ml, 144 mmol) was added. After standing at room temperature for 45 min, triethylamine (20 ml, 143 mmol) was added and the mixture was taken down in vacuo. The residue was stirred with acetone (150 ml) which brought about crystallization of the product; ether (400 ml) was then gradually added with stirring which was continued for 15 min. After standing overnight, the product was collected with acetone–ether (1 : 2) and ether, and dried in vacuo. Yield 26 g (71%) of compound *(S)-XXVII*, m.p. 178–179°C. For $C_{21}H_{19}N_3O_5$ (393·4) calculated: 64·11% C, 4·87% H, 10·86% N; found: 64·35% C, 4·93% H, 10·79% N. ^{1}H NMR ($(CD_3)_2SO$): 3·68 m, 2 H (3′-CH_2); 4·08 dd, 1 H + 4·33 dd, 1 H (1′-CH_2, $J(1', 2') = 2·5$, $J(1'', 2') = 8·5$, J(gem) = −13·5); 5·16 t, 1 H (3′-OH, $J(3', OH) = 5·3$); 5·37 m, 1 H (2′-CH); 7·10–8·25 m, 12 H (arom. + 5-H + 6-H); 11·10 br s, 1 H (NH). $[\alpha]_D^{20}$ −210° (*c* 0·5, dimethylformamide). R_F 0·80 (S3).

1-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine ((*S*)-*XXIt*, HPMPC)

Compound *XVII* (11 ml, 0·1 mol) was added to a stirred suspension of compound *(S)-XXVII* (25 g, 63·5 mmol) in triethyl phosphate (150 ml). After stirring to homogeneity and standing overnight in a stoppered flask, the mixture was poured into ether (1·5 l), filtered through a sintered glass filter and the solid on the filter was washed with ether (500 ml). The still ether-containing product was stirred with a mixture of dioxane (250 ml) and 1M triethylammonium hydrogen carbonate for 1 h. The mixture was evaporated in vacuo, codistilled with ethanol (3 × 100 ml) and the residue dried in vacuo over phosphorus pentoxide. 1M Methanolic sodium methoxide (500 ml) was added and the mixture was refluxed (calcium chloride tube) for 14 h. The mixture was neutralized with Dowex 50 × 8 (H^+-form, prewashed with methanol), made alkaline with triethylamine, filtered and the solid was washed with methanol (1 l) and water (1 l). The filtrate was concentrated in vacuo to about 500 ml, extracted with ether (3 × 100 ml) and the aqueous layer was evaporated to dryness. The residue was codistilled with ethanol (3 × 200 ml) and toluene (3 × 100 ml), and dried over phosphorus pentoxide and dissolved in acetonitrile (400 ml). Bromotrimethylsilane (40 ml, 300 mmol) was added and the mixture was stirred in a stoppered flask at room temperature overnight. The solvent was evaporated in vacuo, the residue was codistilled with acetonitrile (2 × 100 ml), dissolved in 0·4M buffer S5 (300 ml) and made alkaline with triethylamine. The mixture was evaporated, the residue was codistilled with methanol (4 × 200 ml), dissolved in water (100 ml) and applied onto a column of Dowex 50 × 8 (H^+-form, 600 ml). After inorganic acids, water eluted a UV-absorbing fraction which after evaporation in

vacuo and crystallization from water (with addition of 4 volumes of ethanol and then ether to turbidity) afforded monohydrate of compound *(S)-XXXII*, m.p. 243–244°C. Yield 2·2 g (11%). For $C_8H_{12}N_3O_5P.H_2O$ (279·3) calculated: 34·40% C, 5·05% H, 15·05% N, 11·11% P; found: 34·67% C, 5·30% H, 14·97% N, 11·40% P. ^{13}C NMR (D_2O); 59·29 s (1′-CH_2); 65·20 d (P–CH_2, ^1J(P, C) = 143·5); 69·89 d (3′-CH_2, ^2J(P, C) = 6·7); 74·28 d (2′-CH_2, ^3J(P, C) = 3·9); 95·62 s (5-C); 147·63 s (6-C); 158·43 s (2-C); 167·23 s (4-C). $[\alpha]_D^{20}$ −91·1° (*c* 0·5, 0·1M HCl). R_F 0·42 (S4), E_{Up} 0·66, UV (pH 2); 280 (10 300).

After elution of the compound *(S)-XXXII*, the column was washed with 2·5% ammonia. The obtained UV-absorbing eluate was taken down in vacuo and applied onto a column of Dowex 1 × 2 (acetate form, 600 ml). The column was washed with water to remove the salts, and then with 0·5M acetic acid. The UV-absorbing fraction was evaporated, the residue was codistilled with water to remove the acetic acid (4 × 100 ml), dissolved in water (100 ml) and neutralized with sodium hydroxide to pH 7. This solution was passed through a column of Dowex 50 × 8. (Na^+-form, 100 ml) which was then washed with water to drop of UV absorption to the original value. Evaporation in vacuo, codistillation with ethanol, mixing with ethanol–ether and filtration afforded disodium salt of compound *(S)-XXIt* (HPMPC); yield 14 g (68%). For $C_8H_{12}N_3Na_2O_6P$ (323·3) calculated: 13·00% N, 9·60% P; found: 12·86% N, 9·65% P, $[\alpha]_D^{20}$ −108·9° (*c* 0·5, 0·1M HCl). Other parameters of this compound agreed with those for the racemate (Table II).

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