SYNTHESES OF ENANTIOMERIC N-(3-HYDROXY-2-PHOSPHONOMETHOXYPROPYL) DERIVATIVES OF PURINE AND PYRIMIDINE BASES

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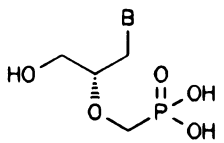
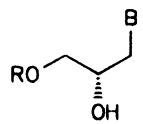
Methods of preparation of N-(3-hydroxy-2-phosphonomethoxypropyl) (HPMP) derivatives of (2*S*)- and (2*R*)-configuration (compounds *I* and *XXVII*, respectively) are described. The general method starts from the corresponding N-(2,3-dihydroxypropyl) derivatives which were converted either into the (*R*)-enantiomers *XIII* by reaction of the base with (*R*)-glycidol butyrate (*XII*) in the presence of cesium carbonate and subsequent methanolysis, or into the (*S*)-enantiomers *XI* by alkylation of the base with (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*V*) in the presence of the same reagent. The amino groups on the heterocyclic base in compounds *XI* and *XIII* were benzoylated by silylation followed by reaction with benzoyl chloride and the obtained N-benzoates *XV* and *XVII* on reaction with trityl chloride afforded the corresponding 3'-O-trityl derivatives *XVI* and *XVIII*. These compounds were condensed with bis(2-propyl) *p*-toluenesulfonyloxymethanephosphonate (*XXIII*) in dimethylformamide in the presence of sodium hydride to give the fully protected diesters *XXIV* and *XXVIII*. These compounds could be selectively acid-hydrolyzed to remove the trityl group only under formation of compounds *XXXV*, or methanolyzed and then acid-hydrolyzed to remove the trityl and N-benzoyl groups and lead to compounds *XXVI* and *XXX*, or treated with bromotrimethylsilane to remove the trityl and 2-propyl group to give phosphonates of the type *XXXI*. All the three types of compounds were then converted into free phosphonates of the (*S*)-series (*I*) and (*R*)-series (*XXVII*). Derivatives of cytosine (*Ia*, *XXVIIa*), adenine (*Ib*, *XXVIIb*), 2,6-diaminopurine (*Ic*, *XXVIIc*) and guanine (*Id*, *XXVIId*) were prepared. Condensation of the partially blocked adenine derivative *XXXV* with the tosyl derivative *XXIII* and subsequent deprotection afforded 9-(*S*)-(2,3-diphosphonomethoxypropyl)adenine (*XLIII*). Reaction of the same compound *XXXV* or its (*R*)-enantiomer *XXXVIII* with diethyl chlorophosphonate, followed by deblocking, afforded 3'-O-phosphoryl derivatives (*S*)-HPMPA (*XXXVII*) and (*R*)-HPMPA (*XL*).

Recently, considerable attention has been paid to the study of biological effects of the so-called HPMP-derivatives, i.e., (*S*)-N-(3-hydroxy-2-phosphonomethoxypropyl) derivatives of heterocyclic (purine and pyrimidine) bases (*I*). These compounds exhibit significant antiviral activity against DNA viruses (for a review see refs[1,2]) which has been exemplified on many in vitro as well as in vivo experimental models[3,4]; some of them show also an antiparasital effects, e.g. against *Plasmodium sp.* (ref.[5]). Several approaches to the preparation of these so-called acyclic nucleotide analogs[6] have been elaborated, most of them based on attaching phosphonomethyl ether functionality to the corresponding N-(2,3-dihydroxypropyl) derivatives *II*, either by transformation of the

presynthesized chloromethanephosphonates with alkali metal hydroxide or methoxide[7,8] or by condensation with diester of *p*-toluenesulfonyloxymethanephosphonic[9,10] or methanesulfonyloxymethanephosphonic[11] acid. Regioselectivity of these procedures must be guaranteed by suitable protection both of the heterocyclic base and the side chain. To this end, the first alternative makes use mainly of acyl (benzoyl) groups which have to be introduced into the position 2′. The procedure consists of two steps and utilizes the selectivity of tritylation in position 3′ of the side chain[8]. Although the first variant was employed successfully in the preparation of gram quantities of N-(3-hydroxy-2-phosphonomethoxypropyl) derivatives, it has several drawbacks: the temporarily introduced trityl functionality must be very acid-labile in order to be easily removable without degradation of the benzoyl ester functionality. The dimethoxytrityl group, mainly used for this purpose, meets these requirements but requires expensive reagents. In the obtained 2-O-benzoyl derivatives the benzoyl group very easily migrates to the neighbouring 3′-hydroxy group in the presence of traces of alkali, leading to a mixture of chloromethanephosphonates and, after the intramolecular etherification step, to a mixture of isomeric phosphonomethyl ethers.



I



II, R = H

III, R = triphenylmethyl

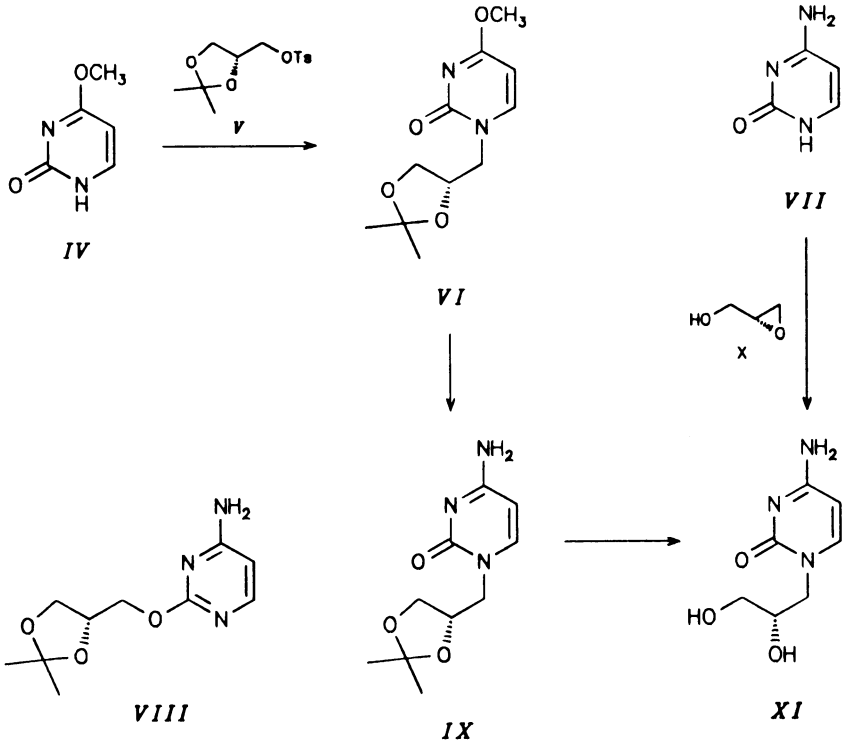
For obvious reasons, the second alternative, introducing of the mentioned functionality by condensation with the organophosphorus synthon in the presence of sodium hydride, does not allow to use acyl group for protection at position 3′ and, on the contrary, requires introduction of an alkali-labile protecting group. A trityl-type group again appears to be the most suitable for this purpose. In one publication[10], the adenine derivative HPMPA was prepared in this way from N^6,3′-ditrityl derivative of 9-(*S*)-(2,3-dihydroxypropyl)adenine. An analogous procedure had to be used also in the preparation of the 3-deazaadenine analog[12]. The apparent simplicity of this monothematic simultaneous protection of hydroxy and amino groups is depreciated by the difficult preparation of such compound which enforces the use of excess reagent, lowering thus the selectivity of substitution at the primary hydroxyl. Under such circumstances, the best way appeared to be the combination of both protecting groups, i.e., an alkali-labile group for the protection of the amine functionality (if present in the molecule) and the unsubstituted trityl group for selective protection of the primary hydroxyl in compounds *II*. In principle, as alkali-labile protecting group one may also use the amidine functionality which is introduced by reaction of the amino derivative with

dimethylformamide dialkyl acetal; this approach was used already earlier by us[9] and later by other authors[13]. However, with some heterocyclic systems (particularly those containing strongly basic amino groups) the simplicity of the transformation of the amino group is obscured by the relatively high lability of the formed amidine in acidic as well as alkaline medium[12]. Therefore, in most cases the group of choice is the N-benzoyl functionality. It can be selectively introduced to an amino group by reaction with chlorotrimethylsilane and benzoyl chloride in pyridine[14] and the obtained products usually can be well crystallized or chromatographed on silica gel. The mentioned combination of protecting groups has only one limitation: as we have found[15], the cleavage of trityl derivatives of N^4-benzoylcytosine nucleosides with acetic acid is accompanied by migration of the benzoyl group to the position N^3 which, as final consequence, leads to uracil derivatives. This danger can be avoided by using a strong mineral acid of low nucleophilicity (e.g. sulfuric acid).

The present study concerns method of laboratory synthesis of N-(3-hydroxy-2-phosphonomethoxypropyl) derivatives of purine and pyrimidine bases (*I*) from N-(2-hydroxy-3-O-trityloxypropyl) derivatives *III* that may be utilized for their preparation on a large scale and in high purity. These principles have been taken over from us by other authors[16]. Particular attention is paid to compounds with proved biological activity: derivatives of cytosine, adenine, guanine and 2,6-diaminopurine. Since some data have appeared indicating that the originally declared enantioselectivity of the antiviral effect of compounds *I* is obviously of only limited validity and depends on the virus[17], we further aimed at the preparation of the compounds in both enantiomeric series in order to investigate the generality of the enantioselectivity in relation to the heterocyclic base as well as to the virus.

The synthesis of (*S*)-N-(2,3-dihydroxypropyl) derivatives was described in detail already several times[8,18 - 20]; in the present paper we describe a new modification of preparing one of the key compounds, 1-(*S*)-(2,3-dihydroxypropyl)cytosine (*XI*) which has been hitherto prepared by ammonolysis of the corresponding 4-methoxy-2-pyrimidone derivative *VI*, obtained from sodium salt of this base (*IV*) by reaction with (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane[8] (*V*). Cytosine (*VII*), which in the form of sodium salt or in the presence of potassium carbonate reacted with this reagent only sluggishly and with preferential formation of the O^2-isomer *VIII*, in the presence of cesium carbonate afforded smoothly and preferentially the desired N^1-isomer *IX*; this observation is supported by an analogous result of alkylation of cytosine with the corresponding mesyl derivative[21]. Although this synthesis does not give higher overall yield of compound *IX* than the mentioned route starting from 4-methoxy-2-pyrimidone (*IV*), it is shorter and simpler. It appears that a rapid and smooth course of cytosine alkylation does not require excess of cesium carbonate as stated for the analogous mesyl derivative[21]. Naturally, even replacement of sodium salt of 4-methoxy-2-pyrimidone (*IV*) by a mixture of the free base and cesium carbonate in the reaction with the

tosyl derivative *V* represents a more advantageous modification of this alkylation reaction. Still better is using cesium carbonate in the alkylation of cytosine with (*R*)-glycidol (*X*) (Scheme 1).

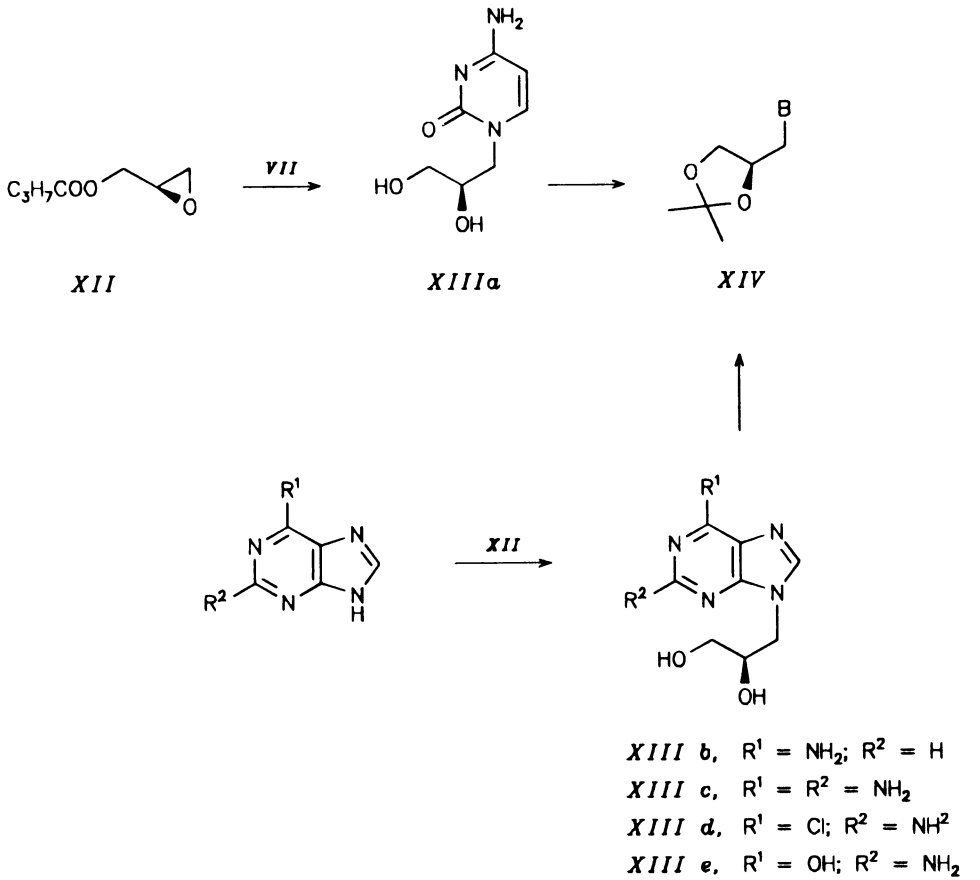


Ts *p*-toluenesulfonyl

SCHEME 1

The reaction with cesium carbonate has been successfully employed also in the preparation of (*R*)-(2,3-dihydroxypropyl) derivatives *XIII* by alkylation of heterocyclic bases with (*R*)-glycidol butyrate ((*R*)-butanoyloxymethyloxirane, *XII*). Reaction of this compound with cytosine (*VII*) gave the acylated intermediate which upon methanolysis afforded the expected N-alkyl derivative *XIIIa*; with analogous ease we obtained the adenine and 2,6-diaminopurine derivatives *XIIIb* and *XIIIc*, respectively. 2-Amino-6-chloropurine afforded predominantly the N^9-(*R*)-(2,3-dihydroxypropyl) derivative *XIIId* which could be easily converted into N^9-(*R*)-(2,3-dihydroxypropyl)guanine *XIIIe* by acid-catalyzed hydrolysis (Scheme 2). Compounds *XIII* were again converted to 2,3-O-isopropylidene derivatives *XIV* by reaction with 2,2-dimethoxypropane.

The enantiomeric N-(2,3-dihydroxypropyl) derivatives *XI* and *XIII* were converted to the N-benzoyl derivatives *XV* and *XVII*. It might seem that these compounds could be obtained directly by condensation of the three-carbon synthon with the N-benzoyl derivative of the heterocyclic base; however, the N-benzoyl group is not entirely stable under conditions of deprotection, being partially cleaved during acid hydrolysis of

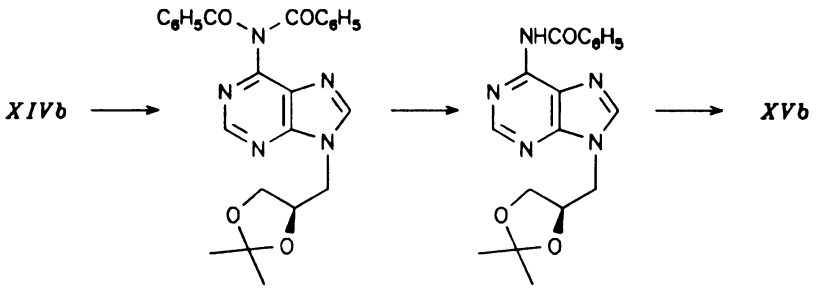


XIII b, $R^1 = NH_2$; $R^2 = H$
XIII c, $R^1 = R^2 = NH_2$
XIII d, $R^1 = Cl$; $R^2 = NH^2$
XIII e, $R^1 = OH$; $R^2 = NH_2$

SCHEME 2

compounds *IX* and, naturally, in methanolysis of intermediates obtained by alkylation of the bases with compound *XII*. Moreover, the course of the benzoylation is often equivocal, leading to perbenzoyl derivatives which then have to be partially debenzoylated. We used this procedure with the adenine derivative *XIV* whose reaction with benzoyl chloride in pyridine gave the N^6-dibenzoyl derivative *XIX*. Treatment of *XIX* with ammonia in aqueous dioxane afforded N^6-benzoyl derivative *XX* which on acid hydrolysis was converted into the desired (*R*)-enantiomer *XVb* (Scheme 3).

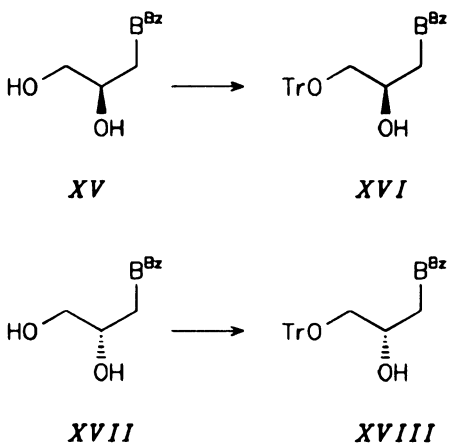
Preparatively preferable is the variant consisting in reaction of compounds *XI* and *XIII* with chlorotrimethylsilane and benzoyl chloride in pyridine (vide supra), even though one has to consider the formation of benzamide and a great amount of salt, so that the N-benzoyl derivatives *XV* and *XVII* usually require chromatographic isolation. Cytosine, adenine and guanine derivatives afforded smoothly the corresponding mono-N-benzoyl derivatives *XVa*, *XVb*, *XVd* and *XVIIa*, *XVIIb*, *XVIId*; as expected, the 2,6-diaminopurine derivative was converted into 2,6-bis(benzoylamino)purine derivative *XVc*. All the thus-obtained compounds were characterized by their NMR spectra.



XIX XX

SCHEME 3

Reaction of benzoyl derivatives *XV* and *XVII* with trityl chloride in dimethylformamide in the presence of a small excess of pyridine, catalyzed with 4-dimethylaminopyridine at elevated temperature, afforded 3-O-trityl derivatives *XVI* and *XVIII* (Scheme 4), the starting compounds for condensation with the organophosphorus



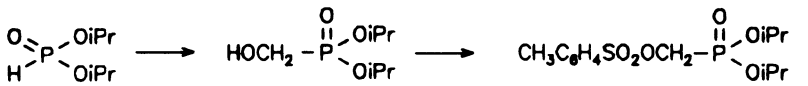
XV XVI

XVII XVIII

Tr triphenylmethyl; B^{Bz} see Scheme 6

SCHEME 4

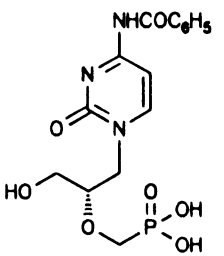
synthon, diester of *p*-toluenesulfonyloxymethanephosphonic acid. Since we found that, analogously to reaction with phosphoric acid triesters, in a side-reaction these compounds are alkylating the heterocyclic base (the amount of the side-product decreasing in the order benzyl > methyl > ethyl), we replaced the hitherto used diethyl ester with the bis(2-propyl) ester; this synthon was prepared from bis(2-propyl) phosphite (*XXI*) which on reaction with paraformaldehyde under catalysis with triethylamine gave hydroxymethanephosphonate *XXII*. The crude intermediate *XXII* was tosylated to give compound *XXIII*, obtained in the crystalline state upon chromatography (Scheme 5).



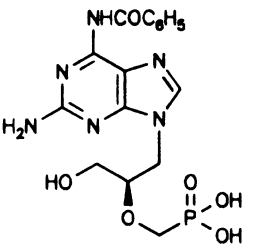
XXI *XXII* *XXIII*

iPr 2-propyl

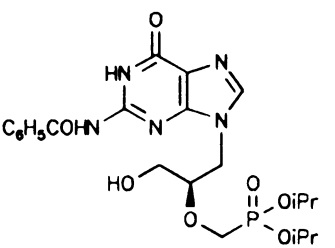
SCHEME 5



XXXI



XXXII



XLI

iPr 2-propyl

The optimum execution of the condensation of both key compounds consists in using a small excess of the synthon *XXIII* relative to the protected intermediate *XVI* or *XVIII*. The reaction is carried out in dimethylformamide by addition of 3 equivalents of sodium hydride (relative to compound *XVI* or *XVIII*) to a mixture of both reactants at a low temperature (−10 °C to −20 °C); the reaction then proceeds at room or elevated temperature and can be monitored by chromatography.

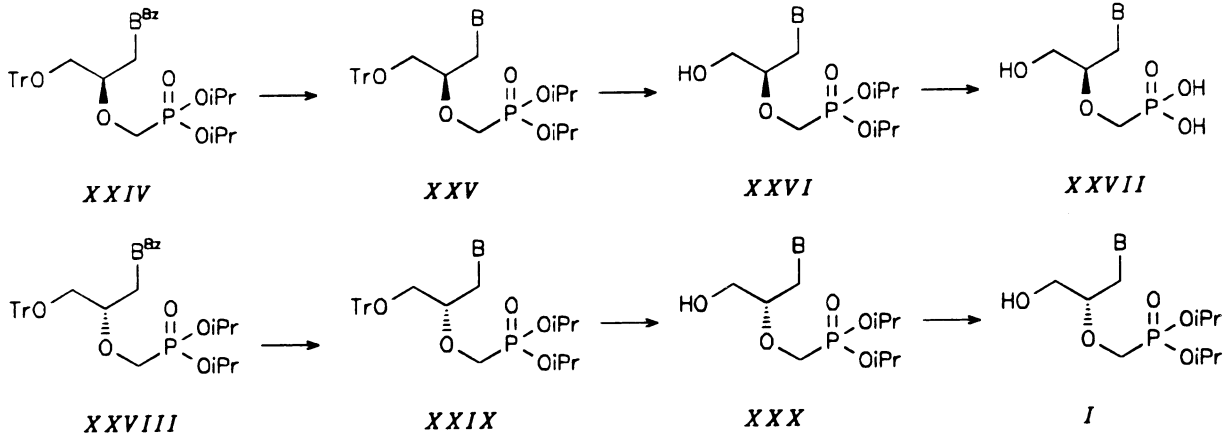
Further work-up of the reaction mixture depends on the intended synthetic utilization of the formed fully protected intermediates *XXIV* and *XXVIII*. In the preparation of the enantiomeric compounds *I* and *XXVII* the reaction mixture was directly subjected first to methanolysis and then to hydrolysis with acetic acid which converted the trityl derivatives *XXIV* and *XXVIII* into the unprotected bis(2-propyl) esters *XXVI* and *XXX*, respectively. This reaction sequence must be observed particularly in the case of cytosine in order to suppress migration of the N^4-benzoyl group (vide supra). N-Debenzoylation of 2,6-diaminopurine derivatives is difficult. Therefore, after the final work-up also the monobenzoyl derivative *XXXII* was isolated from the reaction mixture.

The ester-bonded groups of the phosphonic acid are completely stable under the mentioned conditions (they do not undergo even reesterification), so that bis(2-propyl) esters *XXVI* and *XXX* can be deionized by ion-exchange chromatography and isolated. Alkaline hydrolysis, which proceeds smoothly with methyl and ethyl esters[10], in this case is extremely difficult; although we obtained limited amounts of mono(2-propyl) ester, the only effective method for cleavage of the ester bond in compounds *XXVI* and *XXX* is their reaction with bromotrimethylsilane, followed by hydrolysis[9,10] (Scheme 6).

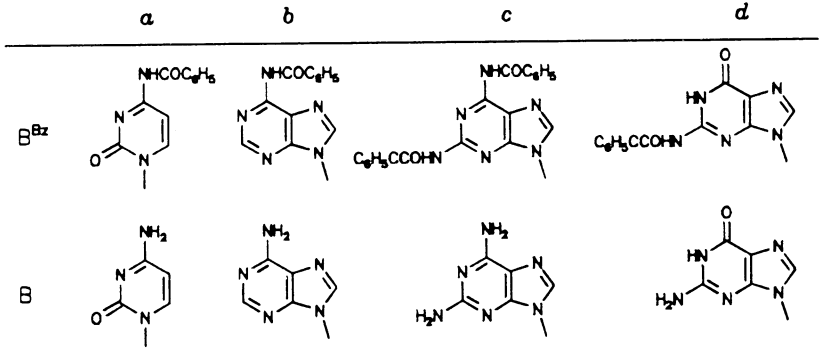
After the usual processing by deionization and ion-exchange chromatography, the described procedure gave the respective enantiomeric (*R*)- and (*S*)-(3-hydroxy-2-phosphonomethoxypropyl) derivatives *XXVII* and *I* in good yields. Their optical purity was verified by HPLC on a chiral phase and was in all cases higher than 98% for the (*S*)-derivatives; for the (*R*)-derivatives *XXVII* the enantiomeric purity limit was 95%, in accord with the enantiomeric purity of the starting compound *XII*.

The high stability of the ester bond in bis(2-propyl) phosphonate esters allows to remove selectively the trityl group from some protected diesters *XXIV* and *XXVIII*. Thus, e.g., the adenine derivatives *XXIVb* and *XXVIIIb* on boiling with 80% acetic acid afforded N-benzoyl derivatives *XXXV* and *XXXVIII* with free hydroxyl group; similarly, N-benzoylguanine derivative *XLI* was prepared from the fully protected starting compound *XXVIIId*.

In the cytosine series, this reaction cannot be used for the reasons already mentioned. On the other hand, it is possible to remove simultaneously the trityl protecting group and the ester-bonded alkyl groups under preservation of the N-benzoyl functionality: the fully protected derivative *XXVIIIa* upon treatment with bromotrimethylsilane in acetonitrile and subsequent work-up in the presence of water gave N^4-benzoyl-(*S*)-HPMPC (*XXXI*). There was no N-benzoyl migration: after debenzoylation by methanolysis in



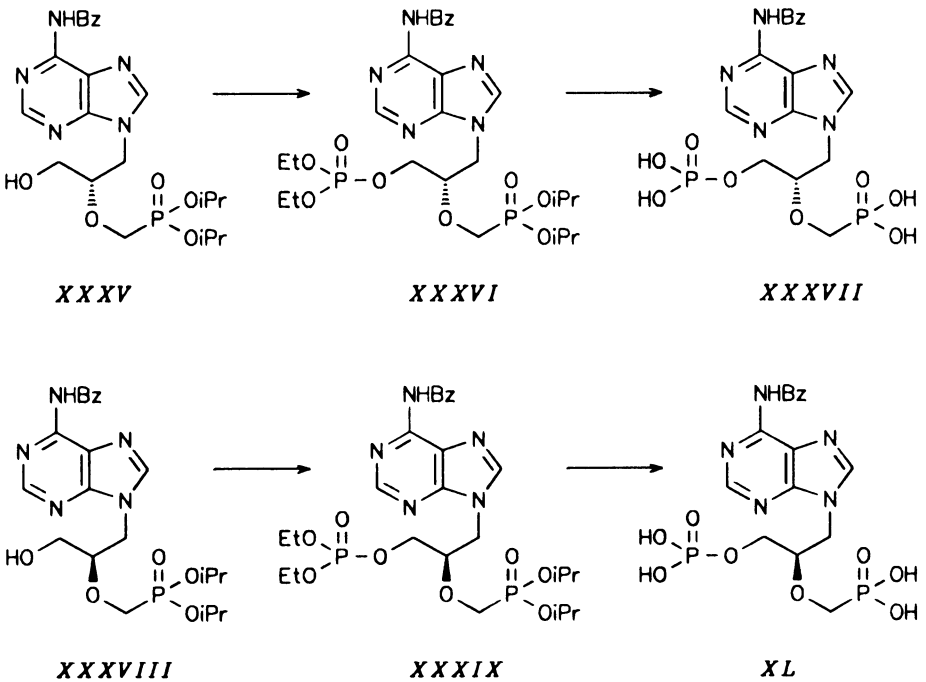
In formulae *I*, *XXIV* – *XXX* : Tr triphenylmethyl; iPr 2-propyl



SCHEME 6

the presence of sodium methoxide, (*S*)-HPMPC (*Ia*) was obtained as the sole product and no uracil derivative was found in the mixture.

The character of thus-obtained intermediates of the type *XXXV* (protected phosphonate functionality and amino group, only one free hydroxyl) makes them suitable for further synthetic utilization. Reaction of enantiomeric adenine derivatives *XXXV* and *XXXVIII* with diethyl chlorophosphate in the presence of a soft base, followed by deprotection of the tetraesters *XXXVI* and *XXXIX* by methanolysis and treatment with bromotrimethylsilane afforded 3'-O-phosphates (*S*)- and (*R*)-HPMPA (*XXXVII* and *XL*, respectively). The aim of their synthesis was, inter alia, to find out whether such compounds can arise in phosphorylation of HPMP derivatives in cells and whether they can act as possible intermediates in transformations of HPMP derivatives into analogs of di- or triphosphates[22,23] (Scheme 7).

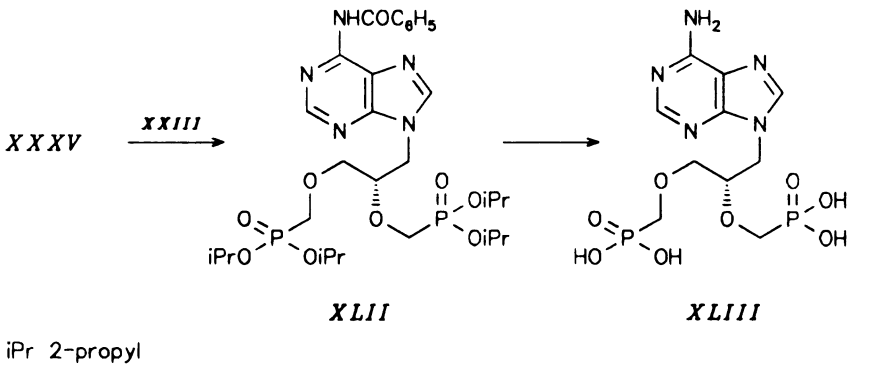


Bz benzoyl; iPr 2-propyl

SCHEME 7

Compound *XXXV* was also used as the starting material for the preparation of a representative of a further, hitherto undescribed, type of acyclic nucleotide analogs, 9-(*S*)-[2,3-bis(phosphonomethoxy)propyl]adenine (*XLIII*). Condensation of compound

XXXV with tosyl derivative *XXIII* in the presence of sodium hydride, followed by methanolysis, afforded the tetraester *XLII* which on treatment with bromotrimethylsilane was converted to 2,3-bis(phosphonomethyl) derivative *XLIII*. This compound was not found among the products of condensation of N^6-benzoyl derivative *XVIIb* (with free vicinal diol group) with an excess of reagent *XXIII*. Analogous derivatives can be obviously more easily prepared according to the procedure described in Scheme 8.



iPr 2-propyl

SCHEME 8

Compound *XLIII* exhibited no antiviral effect against DNA viruses under standard conditions of in vitro essays. Comparison of biological effects of the (*R*)-enantiomers *XXVII* described in this paper will be published elsewhere[24].

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Solvents were evaporated on a rotatory evaporator at 40 °C. Analytical samples were dried at 25 °C and 6.5 Pa for 8 h. Optical rotations were determined on a Perkin–Elmer 141 polarimeter at 25 °C in dimethylformamide or 0.1 M HCl. NMR spectra were measured on a Varian 200 XL instrument in FT mode at 200 MHz (^{1}H spectra) in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. UV absorption spectra were measured on a Pye Unicam 8800 UV-VIS spectrophotometer, the wavelengths of the extrema are given in nm. Thin-layer chromatography was performed on Silufol UV_{254}, column chromatography on Silpearl silica gel (both Kavalier, Votice, Czech Republic). Solvent systems for TLC: S1 chloroform–methanol (95 : 5), S2 chloroform–methanol (9 : 1), S3 chloroform–methanol (4 : 1), S4 chloroform–methanol (7 : 3), S5 benzene–ethyl acetate (7 : 3), S6 ethyl acetate–acetone–ethanol–water (4 : 1 : 1 : 1). Paper chromatography was carried out in the system S7, 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2) (paper Whatman 1). High performance liquid chromatography was performed on 250 × 4 mm or 250 × 17 mm columns packed with Separon SGX C18 (5 μm or 10 μm; Laboratorní přístroje, Prague, Czech Republic), isocratic elution (1 ml/min) with 0.05 M triethylammonium hydrogen carbonate, pH 7.5, containing 5 vol.% of acetonitrile (S8); detection at 254 nm. Paper electrophoresis was done on a Whatman 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate, pH 7.5 (S9).

Chemicals and reagents. Adenine was a Janssen (Belgium) product, 2-amino-6-chloropurine was purchased from Mack company (Germany). Cytosine was obtained from Fluka (Switzerland), bis(2-propyl) phosphite from Strem company (France), (*R*)-glycidol butyrate from Genzyme Fine Chemicals company (United Kingdom) and (*R*)-glycidol was an Arco (U.S.A.) product. 4-Methoxy-2-pyrimidone was prepared according to a published procedure[25]. Dimethylformamide, dichloromethane and acetonitrile were dried by distillation from phosphorus pentoxide and stored over molecular sieves.

Bis(2-propyl) *p*-Toluenesulfonyloxymethylphosphonate (*XXIII*)

Triethylamine (8.4 ml) was added to a stirred mixture of bis(2-propyl) phosphite (96 g) and paraformaldehyde (23 g) and the mixture was heated at 100 °C to complete dissolution of the paraformaldehyde and then for 1 h more. After cooling, acetonitrile (500 ml) and *p*-toluenesulfonyl chloride (121.5 g) were added to the stirred mixture, followed by triethylamine (90 ml) and 4-dimethylaminopyridine (1 g). The mixture was stirred for 24 h, cooled with ice, and triethylammonium hydrogen carbonate (400 ml of 0.4 M solution) was added. After stirring for further 24 h, acetonitrile was evaporated in vacuo at 40 °C and the mixture was extracted with ether (3 × 200 ml). The ethereal extract was dried over sodium sulfate, filtered, the filtrate was concentrated in vacuo and the residue chromatographed in benzene (200 ml) on a column of silica gel (500 ml). Elution with benzene removed impurities of higher R_F (TLC on silica gel plates in chloroform) and the product was eluted with ethyl acetate. After evaporation of the solvent in vacuo, the remaining oil was stirred with light petroleum (300 ml) to solidification, the solid was collected, washed with light petroleum and dried in vacuo. Yield 140 g of bis(2-propyl) *p*-toluenesulfonyloxymethylphosphonate, m.p. 37.5 °C. For $C_{14}H_{23}O_6PS$ (350.4) calculated: 47.98% C, 6.61% H, 8.86% P, 9.15% S; found 48.15% C, 6.80% H, 9.02% P, 9.24% S.

9-(*R*)-(2,3-Dihydroxypropyl)adenine (*XIIIb*)

A) (*R*)-Glycidol butyrate (*XII*; 34 g) was added at 100 °C to a stirred mixture of adenine (27 g, 0.2 mol), potassium carbonate (1.5 g) and dimethylformamide (600 ml). The mixture was heated at 100 °C for 3 h, filtered while hot and washed with dimethylformamide (50 ml). The filtrate was concentrated in vacuo, the residue mixed with 0.05 M sodium methoxide in methanol (500 ml) and the solution set aside overnight. The separated product was collected, washed with ethanol and ether and dried in vacuo; yield 32 g (76%) of 9-(*R*)-(2,3-dihydroxypropyl)adenine, homogeneous according to TLC in S4. An analytical sample was crystallized from methanol with addition of ether. For $C_8H_{11}N_5O_2$ (209.2) calculated: 45.93% C, 5.30% H, 33.48% N; found: 46.15% C, 5.45% H, 33.70% N.

B) A solution of compound *XIVb* (10 g, 40 mmol) in 0.25 M sulfuric acid (150 ml) was allowed to stand overnight at room temperature. After neutralization with saturated barium hydroxide solution, the mixture was heated to 80 °C and filtered while hot. The precipitate was washed with boiling water (1 l), the filtrate was evaporated and the residue codistilled with ethanol and crystallized from 80% ethanol with addition of ether. Yield 7.7 g (92%) of compound *XIIIb*, identical with the product obtained by procedure *A*.

9-(*R*)-(2,2-Dimethyl-1,3-dioxolan-4-ylmethyl)adenine (*XIVb*)

Compound *XIIIb* (prepared from 1.35 g (10 mmol) of adenine according to procedure *A*) was mixed with dimethylformamide (40 ml), 2,2-dimethoxypropane (40 ml) and 6 M HCl in DMF (5 ml). The mixture was stirred overnight with exclusion of moisture, made alkaline with triethylamine, the solid was filtered off, washed with acetone, and the filtrate was evaporated. Column chromatography on silica gel (150 ml) in chloroform (the product was eluted with chloroform–methanol (9 : 1)), followed by crystallization from methanol with addition of ether, afforded 1.1 g (44%) of compound *XIVb*, m.p. 221 °C; R_F 0.50 (S2); $[\alpha]_D$

+22.3° (*c* 0.5, dimethylformamide). For $C_{11}H_{15}N_5O_2$ (249.3) calculated: 53.00% C, 6.06% H, 28.10% N; found: 53.15% C, 5.99% H, 27.85% N.

N^6,N^6-Dibenzoyl-9-(*R*)-(2,2-dimethyl-1,3-dioxolan-4-ylmethyl)adenine (*XIX*)

4-Dimethylaminopyridine (0.6 g) and benzoyl chloride (12 ml, 14.5 g, 103 mmol) were added to an ice-cooled mixture of isopropylidene derivative *XIVb* (12.5 g, 40 mmol) and pyridine (180 ml). The mixture was stirred at 0 °C for 2 h and at room temperature overnight. After addition of methanol (10 ml) the mixture was concentrated, the residue dissolved in ethyl acetate (200 ml), washed with water (50 ml) and the layers were quickly separated before the product began to precipitate. The organic phase was concentrated in vacuo and the residue was boiled briefly with ethanol (250 ml). After cooling, the solid was collected, washed with ethanol and ether, and dried in vacuo, yield 15.3 g (84%) of dibenzoyl derivative *XIX*, m.p. 232 °C; $[\alpha]_D$ +13.3° (*c* 0.5, dimethylformamide). For $C_{25}H_{23}N_5O_4$ (457.5) calculated: 65.63% C, 5.07% H, 15.31% N; found: 65.29% C, 5.13% H, 15.17% N. ^{1}H NMR spectrum: 1.33 s, 6 H (isopropylidene); 3.70 dd, 1 H (J(3",2') = 5.6, J(gem) = 8.8) and 4.13 dd, 1 H (J(3',2') = 6.0) (3'-CH_2); 4.15 – 4.60 m, 3 H (1-CH_2 + 2'-CH); 8.19 + 8.66 2 × s, 2 H (H-2 and H-8); 7.20 – 7.90 m, 10 H (aromatic H).

N^6-Benzoyl-9-(*R*)-(2,3-dimethyl-1,3-dioxolan-4-ylmethyl)adenine (*XX*)

Concentrated ammonia (70 ml) was added to a solution of compound *XIX* (15 g, 33 mmol) in dioxane (360 ml). The mixture was stirred for 30 min at room temperature, the solvent evaporated and the residue codistilled with dioxane (2 × 100 ml). Crystallization from ethanol with addition of light petroleum afforded 11.7 g (quantitative yield) of compound *XX*, m.p. 125 – 126 °C; $[\alpha]_D$ +21.8° (*c* 0.5, dimethylformamide). For $C_{18}H_{19}N_5O_3$ (353.4) calculated: 61.01% C, 5.41% H, 19.77% N; found: 60.34% C, 5.55% H, 19.55% N.

N^6-Benzoyl-9-(*R*)-(2,3-dihydroxypropyl)adenine (*XVb*)

A) From compound *XX*. A solution of compound *XX* (14.4 g, 33 mmol) in a mixture of dioxane (100 ml) and 0.25 M sulfuric acid (100 ml) was heated at 80 °C for 1 h, cooled, neutralized with triethylamine and the solvent was evaporated in vacuo. The residue was stirred with chloroform (200 ml) and water (100 ml), the solid was collected, washed with chloroform and dried in vacuo, yield 7.8 g (75%) of compound *XVb*, m.p. 202 °C; $[\alpha]_D$ +28.6° (*c* 0.5, dimethylformamide). For $C_{15}H_{15}N_5O_3$ (313.3) calculated: 57.50% C, 4.82% H, 22.36% N; found: 57.64% C, 4.86% H, 22.44% N. ^{1}H NMR spectrum: 3.46 m, 2 H (3'-CH_2); 3.95 m, 1 H (2'-CH); 4.19 br d and 4.47 br d, 2 H (1'-CH_2); 4.95 and 5.20 2 × br s, 2 × 1 H (OH); 8.75 and 8.42 2 × s, 2 × 1 H (H-2 and H-8); 11.11 br s, 1 H (NH); 7.37 – 8.20 m, 5 H (aromatic H).

B) From compound *XIIIb*. A suspension of compound *XIIIb* (32 g, 0.15 mol) in pyridine (200 ml) was evaporated in vacuo, the residue was dissolved in pyridine (800 ml) and chlorotrimethylsilane (108 ml) was added. The mixture was stirred for 1 h, benzoyl chloride (97 ml) was added and stirring was continued for further 2 h. The reaction mixture was cooled with ice and ice-cold water (166 ml) and concentrated aqueous ammonia (370 ml) were successively added. After stirring for 30 min at 0 °C the mixture was concentrated in vacuo and the residue partitioned between water (400 ml) and ethyl acetate (180 ml). The separated crystalline product *XVb* was collected on filter, washed with water and ethyl acetate, and dried. Yield 9 g of chromatographically pure benzoyl derivative *XVb*, R_F 0.30 (S2). The residue was evaporated in vacuo, codistilled with ethanol (2 × 100 ml), stirred with ethanol (250 ml) for 16 h, and the separated salts were filtered off. After evaporation, the residue was column chromatographed on silica gel (500 ml) in chloroform. The corresponding fractions (elution with chloroform–ethanol 9 : 1) were combined, evaporated, and the residue was crystallized from ethanol with addition of ether; yield 27 g of the same product. The total yield amounted to 75% of product identical with that obtained by procedure *A*.

N^6-Benzoyl-9-(*R*)-(2-hydroxy-3-triphenylmethoxypropyl)adenine (*XVIb*)

A suspension of of compound *XVb* (4.8 g, 15 mmol) in pyridine (50 ml) was evaporated in vacuo, the residue was codistilled with pyridine (2 × 50 ml) and dissolved in dimethylformamide (50 ml) and pyridine (25 ml). Trityl chloride (6.2 g) and 4-dimethylaminopyridine (1 g) were added and the mixture was stirred at 50 °C for 20 h under exclusion of moisture. Methanol (20 ml) was added and after 30 min the solvent was evaporated in vacuo. The residue was dissolved in chloroform (500 ml), the solution was washed with saturated solution of sodium hydrogen carbonate (2 × 100 ml), water (100 ml), and dried over magnesium sulfate. After evaporation in vacuo, the residue was stirred with acetone (50 ml), light petroleum (150 ml) was added, and the product was collected, washed with light petroleum and dried; yield 5.7 g (66%) of the trityl derivative *XVIb*, m.p. 214 °C, $[\alpha]_D$ +15.5° (*c* 0.5, dimethylformamide). For $C_{34}H_{29}N_5O_3$ (555.6) calculated: 73.55% C, 5.26% H, 12.61% N; found: 73.42% C, 5.48% H, 12.94% N. ^{1}H NMR spectrum: 2.94 dd, 1 H (*J*(3",2') = 5.9, *J*(gem) = 9.5) and 3.07 dd, 1 H (*J*(3',2') = 5.1) (3'-CH_2); 4.16 m, 1 H (2'-CH); 4.27 dd, 1 H (*J*(1",2') = 8.1, *J*(gem) = 13.9) and 4.46 dd, 1 H (*J*(1',2') = 3.9) (1'-CH_2); 5.50 br s, 1 H (3-OH); 8.72 s, 1 H and 8.36 s, 1 H (H-2 and H-8); 11.2 br s, 1 H (NH); 7.10 – 7.50, 15 H, 7.50 – 7.70, 3 H, 8.05, 2 H (aromatic H).

9-(*R*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine (*XXVIIb*)

A mixture of compound *XVIb* (5.50 g, 10 mmol), tosyl derivative *XXIII* (4.2 g, 12 mmol) and dimethylformamide (40 ml) was cooled to −15 °C and 1.4 g of 60% sodium hydride dispersion (0.84 g, 35 mmol) was added. The mixture was stirred at −10 °C for 1 h and then at room temperature for 72 h. Acetic acid (1.5 ml) was added, the solvent was evaporated at 40 °C and 13 Pa and the residue was stirred and refluxed with 80% acetic acid (60 ml) for 30 min. The mixture was taken down, the residue codistilled with toluene (2 × 25 ml), dissolved in chloroform and washed successively (25 ml each) with water, saturated sodium hydrogen carbonate solution and water, and dried over magnesium sulfate. After evaporation, the remaining diester *XXXVII* (2.3 g, 47%) was set aside with 0.1 M sodium methoxide in methanol (200 ml) for 16 h and then neutralized by addition of Dowex 50 X 8 (H^+ form). The suspension was made alkaline with triethylamine, filtered, the solid washed with methanol, the filtrate evaporated in vacuo, and the residue dried and dissolved in acetonitrile (50 ml). Bromotrimethylsilane (5 ml) was added and the mixture was allowed to stand at room temperature for 3 days. After evaporation in vacuo, the residue was worked up as described for compound *XXVIIa*, affording 1.4 g (44%, calculated on the trityl derivative *XVIb*) of monohydrate of compound *XXVIIb*, m.p. 262 °C; $[\alpha]_D$ +25.0° (*c* 0.5, 0.1 M HCl). For $C_9H_{14}N_5O_5P \cdot H_2O$ (321.2) calculated: 33.65% C, 5.02% H, 21.79% N, 9.65% P; found: 33.44% C, 4.86% H, 21.93% N, 9.54% P.

1-(*R*)-(2,3-Dihydroxypropyl)cytosine (*XIIIa*)

Compound *XII* (52 g) was added at 100 °C to a stirred mixture of cytosine (33.3 g, 0.3 mol), cesium carbonate (3 g) and dimethylformamide (600 ml). The mixture was stirred at 100 °C for 4 h and then the solvent was evaporated in vacuo. The residue was codistilled with toluene (2 × 100 ml) and allowed to stand with 0.05 M methanolic sodium methoxide (500 ml) for 16 h. The separated crystalline product *XIIIa* was collected, washed with ethanol, ether, and dried in vacuo; yield 39 g of product containing (TLC in S6) a small amount of cytosine. This product was used in the next step without further purification.

The filtrate was evaporated in vacuo and the residue was deionized on a column of Dowex 50 X 8 (H^+ form). After elution with water to disappearance of UV absorption and acidity, the product was eluted with 2% aqueous ammonia. The UV-absorbing fraction was evaporated and the residue was crystallized from 80% aqueous ethanol with addition of ether to give further 4.5 g of chromatographically homogeneous

XIIIa; R_F 0.17 (cytosine 0.20) in S6. M.p. 186 – 187 °C; $[\alpha]_D$ +37.2° (*c* 0.5, dimethylformamide). For $C_9H_{11}N_3O_3$ (185.2) calculated: 45.39% C, 5.99% H, 22.69% N; found: 45.76% C, 6.12% H, 22.77% N.

1-(*R*)-(2,2-Dimethyl-1,3-dioxolan-4-ylmethyl)cytosine (*XIVa*)

A mixture of compound *XIIIa* (prepared from 1.1 g (10 mmol) of cytosine as described above), dimethylformamide (50 ml), 2,2-dimethoxypropane (50 ml) and 6 M HCl in dimethylformamide (5 ml) was stirred for 24 h, made alkaline with triethylamine, filtered, the solid was washed with acetone and the filtrate evaporated. The residue was crystallized from ethanol to give 1.34 g (60%) of compound *XIVa*, m.p. 245 °C; $[\alpha]_D$ +31.3° (*c* 0.5, dimethylformamide). For $C_{10}H_{15}N_3O_3$ (225.3) calculated: 53.32% C, 6.71% H, 18.66% N; found: 53.21% C, 6.52% H, 18.44% N.

N^1-Benzoyl-1-(*R*)-(2,3-dihydroxypropyl)cytosine (*XVa*)

A suspension of compound *XIIIa* (11.7 g, 63 mmol) in pyridine was evaporated in vacuo, the residue was suspended in pyridine (300 ml), and chlorotrimethylsilane (42.5 ml) was added. After stirring for 1 h, benzoyl chloride (38 ml) was added and the stirring was continued for 2 h. The mixture was cooled to 0 °C and water (66 ml), followed by concentrated aqueous ammonia (145 ml), was added dropwise with stirring which was continued for 30 min at 0 °C. After evaporation of the solvent in vacuo, the residue was crystallized from water to give 14.5 g (79%) of practically pure compound *XVa*. An analytical sample was obtained by crystallization from ethanol with addition of ether; m.p. 218 °C; $[\alpha]_D$ +71.4° (*c* 0.5, dimethylformamide). For $C_{14}H_{15}N_3O_4$ (289.3) calculated: 58.12% C, 5.23% H, 14.53% N; found: 58.16% C, 5.18% H, 14.39% N.

N^1-Benzoyl-9-(*R*)-(2-hydroxy-3-triphenylmethoxypropyl)cytosine (*XVIa*)

A mixture of compound *XVa* (13.6 g, 47 mmol), trityl chloride (13.5 g), 4-dimethylaminopyridine (1 g), dimethylformamide (100 ml) and pyridine (40 ml) was stirred at 50 °C for 40 h. Methanol (10 ml) was added and after 30 min the solvents were evaporated in vacuo. The residue was dissolved in chloroform (500 ml) and the solution was washed with saturated sodium hydrogen carbonate solution (3 × 100 ml) and water (100 ml). After drying over magnesium sulfate and evaporation of the solvent, the residue was codistilled in vacuo with toluene (2 × 100 ml), dissolved in benzene (100 ml) and this solution was added dropwise under stirring to light petroleum (700 ml). The separated product was collected, washed with light petroleum and dried in vacuo. Yield 11.8 g (40%) of compound *XVIa*, m.p. 154 °C; $[\alpha]_D$ +82.6° (*c* 0.5, dimethylformamide); R_F 0.78 (S2). For $C_{43}H_{29}N_3O_4$ (651.7) calculated: 79.24% C, 4.49% H, 6.45% N; found: 79.32% C, 4.70% H, 6.30% N.

Evaporation of the filtrate in vacuo and column chromatography of the residue on silica gel (300 ml) in chloroform afforded another 7.4 g (25%) of compound *XVIa*.

9-(*R*)-(3-Hydroxy-2-phosphonomethoxypropyl)cytosine (*XXVIIa*)

A mixture of compound *XVIa* (11.8 g, 18.7 mmol) and compound *XXIII* (8.3 g, 23.7 mmol) was codistilled with dimethylformamide (2 × 50 ml) at 40 °C and 13 Pa and then dissolved in dimethylformamide (70 ml). The solution was cooled to −10°C, sodium hydride (2.3 g of 60% dispersion in paraffin, i.e., 57.5 mmol) was added and the mixture was stirred at room temperature for 2 days. After addition of methanol (200 ml), the solution was set aside for 16 h, neutralized with Dowex 50 X 8 (H^+ form) and made alkaline with triethylamine. The Dowex was filtered off, washed with methanol (200 ml) and the filtrate was evaporated in vacuo. The residue was boiled with 80% acetic acid (150 ml) for 45 min, filtered and the filtrate was taken down. After addition of water (300 ml), the mixture was extracted with ether (3 × 100 ml), the

aqueous phase was evaporated and the residue deionized on a column of Dowex 50 X 8 (H^+ form, 200 ml). After washing the column with water to disappearance of the UV absorption, the product was eluted with dilute (1 : 10) aqueous ammonia. The UV-absorbing fraction was stripped of the solvent and the residue was codistilled with ethanol (2 × 50 ml) and dried in vacuo. The amorphous residue was allowed to stand overnight with bromotrimethylsilane (20 ml) in acetonitrile (100 ml) under exclusion of moisture. After evaporation in vacuo, the residue was codistilled with acetonitrile (50 ml) and dissolved in water (100 ml). After standing for 30 min the mixture was made alkaline with ammonia and the solvent was evaporated. The residue was deionized as described above on a column of Dowex 50 X 8 (H^+ form, 200 ml), the ammonia eluate was taken down and the residue was chromatographed on a column of Dowex 1 X 2 (acetate form, 200 ml); the column was first washed with water and then the product was eluted with 0.2 M acetic acid. The UV-absorbing eluate was concentrated, the residue codistilled with water (3 × 50 ml) and then crystallized from water with addition of ethanol (3 parts) and ether to turbidity. After cooling, the separated product was collected, washed with ether and dried in vacuo; yield 5.1 g (91%) of compound *XXVIIa* (monohydrate), m.p. 278 °C; $[\alpha]_D$ +110.5° (*c* 0.5, 0.1 M HCl). For $C_8H_{11}N_3O_6P \cdot H_2O$ (297.3) calculated: 32.32% C, 5.43% H, 14.14% N, 10.44% P; found: 32.12% C, 5.58% H, 14.42% N, 10.63% P. R_{Up} 0.76.

2-Amino-6-chloro-9-(*R*)-(2,3-dihydroxypropyl)purine (*XIIId*)

A mixture of 2-amino-6-chloropurine (8.5 g, 50 mmol), compound *XII* (8.65 g, 60 mmol), cesium carbonate (0.75 g) and dimethylformamide (125 ml) was stirred at 100 °C for 1 h. The solvent was evaporated under diminished pressure, the residue was codistilled with toluene (2 × 50 ml) and allowed to stand with methanolic 0.02 M sodium methoxide (250 ml) at room temperature overnight. The solution was concentrated, mixed with ethanol (125 ml) and acetone (125 ml), and the precipitate was filtered and washed with acetone (100 ml). The filtrate was evaporated, treated with water (100 ml) and applied onto a column of Dowex 50 X 8 (H^+ form, 300 ml). The column was washed with water to drop in UV absorption and acidity of the eluate and then the Dowex was suspended in water (300 ml). To the obtained suspension aqueous ammonia was added to adjust the mixture to pH 10. The ion exchanger was filtered off, washed with water, the filtrate was evaporated and the residue codistilled with ethanol (2 × 50 ml). The product was dissolved in methanol (200 ml), adsorbed on silica gel (60 g), dried, slurried in chloroform and applied onto a column of silica gel (400 ml). Elution with methanol–chloroform (1 : 9) afforded a product which was precipitated with ether, filtered and dried; yield 5.0 g (41%) of compound *XIIId*, m.p. 205 °C; $[\alpha]_D$ +67.6° (*c* 0.5, dimethylformamide). For $C_8H_{10}ClN_5O_2$ (243.7) calculated: 39.43% C, 4.14% H, 14.55% Cl, 28.75% N; found: 39.13% C, 3.99% H, 14.26% Cl, 28.93% N. ^{1}H NMR spectrum: 3.33 dt, 1 H (J(3",2') = 5.8, J(3',OH) = 5.8, J(gem) = 11.2) and 3.41 dt, 1 H (J(3',2' = 5.2) (3'-CH_2); 3.82 m, 1 H (ΣJ = 28.7) (2'-CH); 3.92 dd, 1 H (J(1",2') = 8.6, J(gem) = 13.8) and 4.20 dd, 1 H (J(1',2') = 3.6) (1'-CH_2); 4.83 t, (J = 5.8) and 5.11 d, (J = 5.0), 2 × 1 H (OH); 6.89 br s, 2 H (2-NH_2); 8.09 s, 1 H (H-8).

9-(*R*)-(2,3-Dihydroxypropyl)guanine (*XIIIe*)

A solution of compound *XIIId* (4.5 g, 18.8 mmol) in 1 M hydrochloric acid (200 ml) was refluxed for 2 h, cooled and neutralized with aqueous ammonia. After evaporation, the residue was crystallized from water to give 3.7 g (87%) of compound *XIIIe*, not melting up to 300 °C; $[\alpha]_D$ +40.6° (*c* 0.5, dimethylformamide). For $C_8H_{11}N_5O_3$ (225.2) calculated: 42.66% C, 4.92% H, 31.10% N; found: 42.60% C, 4.75% H, 31.30% N. ^{1}H NMR spectrum: 3.29 dt, 1 H (J(3",2') = 5.8, J(3',OH) = 5.5, J(gem) =11.0) and 3.37 dt, 1 H (J(3',2') = 5.2) (3'-CH_2); 3.76 m, 1 H (ΣJ = 27.2) (2'-CH); 3.82 dd, 1 H (J(1",2') = 8.0, J(gem) = 13.7) and 4.08 dd, 1 H (J(1',2') = 3.0) (1'-CH_2); 4.81 t, (J = 5.5) and 5.09 d, (J = 5.2) 2 × 1 H (OH); 6.49 br s, 2 H (2-NH_2); 7.62 s, 1 H (H-8); 10.7 br s, 1 H (NH).

N^2-Benzoyl-9-(*R*)-(2,3-dihydroxypropyl)guanine (*XVd*)

A suspension of compound *XIIIc* (3.7 g, 16.5 mmol) in pyridine (50 ml) was evaporated in vacuo. This procedure was repeated twice more and the residue was resuspended in pyridine (100 ml). Chlorotrimethylsilane (13.7 ml) was added and, after stirring for 1 h, benzoyl chloride (10.7 ml) was added and stirring was continued for 2 h. After cooling with ice, the mixture was decomposed with ice-cold water (17 ml) followed by aqueous ammonia (38 ml), stirred for 30 min at 0 °C and evaporated. The residue was treated with water (150 ml) and chloroform (60 ml), the product was filtered, washed with chloroform, acetone and ether and dried. Yield 5.0 g (91%) of compound *XVd*, m.p. 141 °C; $[\alpha]_D$ +47.3° (*c* 0.5, dimethylformamide). For $C_{15}H_{15}N_5O_4$ (329.3) calculated: 54.70% C, 4.59% H, 21.27% N; found: 54.48% C, 4.60% H, 20.97% N. ^{1}H NMR spectrum: 3.35 m, 1 H (3'-CH$_2$); 3.86 m, 1 H (2'-CH); 3.99 dd, 1 H (*J*(1'',2') = 8.6, *J*(gem) = 13.4) and 4.27 dd, 1 H (*J*(1',2') = 3.2) (1'-CH$_2$); 4.88 t, (*J*(3',OH) = 5.0 and 5.17 d, (*J*(2',OH) = 5.2), 2 × 1 H (OH); 7.94 s, 1 H (H-8); 11.10 br s, 1 H (NH); 7.95 – 8.10 m, 2 H and 7.50 – 7.70 m, 3 H (aromatic H).

N^2-Benzoyl-9-(*R*)-(2-hydroxy-3-triphenylmethoxypropyl)guanine (*XVId*)

A solution of compound *XVd* (5.0 g (15 mmol), trityl chloride (7 g) and 4-dimethylaminopyridine (0.5 g) in a mixture of dimethylformamide (60 ml) and pyridine (20 ml) was stirred at 60 °C for 24 h under exclusion of moisture. Methanol (5 ml) was added and after stirring for 20 min the solvents were evaporated. The residue was dissolved in chloroform, the solution washed with water (2 × 50 ml) and dried over magnesium sulfate. After evaporation of the solvent, the residue was stirred with ether (100 ml), the separated product was collected, washed with ether and dried; yield 7.6 g (89%) of chromatographically homogeneous (S1) compound *XVId*, m.p. 146 °C, $[\alpha]_D$ +24.2° (*c* 0.5, dimethylformamide). For $C_{34}H_{29}N_5O_4$ (571.6) calculated: 71.44% C, 5.11% H, 12.25% N; found: 71.79% C, 5.20% H, 12.32% N.

Bis(2-propyl) N^2-Benzoyl-9-(*R*)-(2-phosphonomethoxy-3-triphenylmethoxypropyl)guanine (*XXIVd*)

A mixture of compound *XVId* (7.5 g, 13 mmol) and tosyl derivative *XXIII* (5.2 g, 14.9 mmol) was codistilled with dimethylformamide (3 × 25 ml) at 40 °C and 13 Pa, the residue was dissolved in dimethylformamide (40 ml), cooled to −10 °C and mixed with sodium hydride (1.6 g, 40 mmol, of 60% dispersion in paraffin). The mixture was allowed to warm to room temperature and stirred under exclusion of moisture at 80 °C for 16 h. Methanol (200 ml) was added, the mixture was immediately neutralized with Dowex 50 X 8 (H^+ form), the Dowex was filtered off, washed with methanol and the solvent was evaporated. Column chromatography on silica gel (300 ml) in chloroform afforded 6.2 g (64%) of compound *XXIVd*, m.p. 107 – 109 °C; R_F 0.45 (S3). For $C_{41}H_{44}N_5O_7P$ (749.9) calculated: 65.67% C, 5.91% H, 9.34% N, 4.14% P; found: 65.34% C, 5.78% H, 9.08% N, 4.31% P. ^{1}H NMR spectrum: 1.12 + 1.13 + 1.16 + 1.17 4 × d, 4 × 3 H (*J*(CH$_3$,CH) = 6.1) (CH$_3$); 3.00 dd, 1 H (*J*(3'',2') = 5.1, *J*(gem) = 10.5) and 3.15 dd, 1 H (*J*(3',2') = 3.9) (3'-CH$_2$); 3.78 dd, 1 H (*J*(P,CH) = 9.8, *J*(gem) = 13.4) and 3.81 dd, 1 H (*J*(P,CH) = 9.5) (P-CH$_2$); 3.96 m, 1 H (*J* = 20) (2'-CH); 4.27 dd, 1 H (*J*(1'',2') = 4.5, *J*(gem) = 14.5) and 4.35 dd, 1 H (*J*(1',2') = 6.5) (1'-CH$_2$); 4.53 m, 2 H (P-OCH); 4.81 t (*J* = 5.5) and 5.09 d, (*J* = 5.2), 2 × 1 H (OH); 6.49 br s, 2 H (2-NH$_2$); 7.77 s, 1 H (H-8); 7.10 – 7.90 (aromatic H).

9-(*R*)-(3-Hydroxy-2-phosphonomethoxypropyl)guanine (*XXVIId*)

A solution of compound *XXIVd* (6.2 g, 8.3 mmol) in 0.1 M methanolic sodium methoxide (200 ml) was allowed to stand overnight and then neutralized with Dowex 50 X 8 (H^+ form). The Dowex was filtered, washed with methanol and the solvent was evaporated, the residue was boiled with 80% acetic acid for 30 min, water (200 ml) was added and the mixture was extracted with ether (3 × 50 ml). The aqueous phase was concentrated and the residue codistilled with ethanol (3 × 50 ml) and dried in vacuo. Acetonitrile

(40 ml) and bromotrimethylsilane (4 ml) were added, the solution was set aside at room temperature overnight and the solvent was evaporated. The residue was dissolved in water (50 ml) and made alkaline with ammonia. After evaporation, the residue was applied onto a column of Dowex 50 X 8 (H^+ form; 100 ml) which was washed with water. After washing out the salts the product (longer retention time) was eluted. The appropriate fraction was evaporated and the product crystallized from water with addition of 3 volumes of ethanol. Yield 1.7 g (64%) of compound *XXVIId*, m.p. >260 °C; $[\alpha]_D$ +67.6° (*c* 0.5, 0.1 M HCl). For $C_9H_{14}N_5O_6P$ (319.3) calculated: 33.85% C, 4.42% H, 21.94% N, 9.72% P; found: 32.85% C, 4.58% H, 21.97% N, 9.54% P.

2,6-Diamino-9-(*R*)-(2,3-dihydroxypropyl)purine (*XIIIc*)

A solution of (*R*)-glycidol butyrate (*XII*; 8.65 g, 60 mmol) in dimethylformamide (20 ml) was added to a stirred mixture of 2,6-diaminopurine (7.5 g, 50 mmol), cesium carbonate (1 g) and dimethylformamide (80 ml), preheated to 100 °C. The mixture was stirred at 100 °C for 4 h (quantitative reaction), filtered while hot and the filtrate concentrated in vacuo. The residue was codistilled with toluene (2 × 100 ml) and set aside with 0.1 M methanolic sodium methoxide (200 ml) overnight. The crystalline product was collected, washed with methanol, ether, and dried; yield 7.5 g (67%) of compound *XIIIc* of purity higher than 98% (HPLC in S8). An analytical sample was crystallized from water, m.p. 240 °C. For $C_8H_{12}N_6O_2$ (224.2) calculated: 42.85% C, 5.40% H, 37.49% N; found: 42.55% C, 5.39% H, 37.75% N. ^{1}H NMR spectrum: 3.24 pent, 1 H (ΣJ = 23.0) + 3.35 pent, 1 H (ΣJ = 22.0) (3'-CH_2); 3.77 m, 1 H (ΣJ = 27.8) (2'-CH); 3.84 dd, 1 H (J(1",2') = 7.6, J(gem) = 13.9) and 4.10 dd, 1 H (J(1',2') = 3.7) (1'-CH_2); 4.91 t, (J(3',OH) = 5.7) and 5.46 d, (J(2',OH) = 5.1), 2 × 1 H (OH); 5.94 br s, 2 H and 6.70 br s, 2 H (NH_2); 7.62 s, 1 H (H-8). After exchange: 3.26 dd, 1 H (J(3",2') = 6.1, J(gem) = 11.2) and 3.35 dd, 1 H (J(3',2') = 5.1) (3'-CH_2); 3.77 m, 1 H (ΣJ = 22.7) (2'-CH).

2',3'-O-Isopropylidene derivative (XIVc), m.p. 232 – 233 °C; $[\alpha]_D$ +7.4° (*c* 0.5, dimethylformamide). For $C_{11}H_{16}N_6O_2$ (264.2) calculated: 49.98% C, 6.11% H, 31.80% N; found: 49.76% C, 5.98% H, 31.81% N.

2,6-Bis(benzoylamino)-9-(*R*)-(2,3-dihydroxypropyl)purine (*XVc*)

A suspension of compound *XIIIc* (6.7 g, 30 mmol) in pyridine (100 ml) was evaporated and the residue stirred with pyridine (140 ml) and chlorotrimethylsilane (25 ml, 200 mmol) at room temperature for 1 h. Benzoyl chloride (18.6 ml, 160 mmol) was added, the mixture was stirred at room temperature for 2 h and cooled with ice. Water (30 ml) and concentrated ammonia (70 ml) were successively added and after 30 min at 0 °C the mixture was concentrated. The residue was codistilled with ethanol (4 × 200 ml) and stirred with ethanol (300 ml). The undissolved inorganic salts were filtered off, the filtrate was evaporated and the residue extracted with boiling chloroform (2 × 200 ml). The chloroform extract contained the product (R_F 0.42 in S2) whereas the insoluble portion was benzamide. After filtration, the chloroform was evaporated and the residue crystallized from ethanol, affording 4.1 g of chromatographically pure product *XVc*; chromatography of the mother liquor on a column of silica gel (200 g) and crystallization under the same conditions as above gave further 3.3 g of the same product, the total yield being thus 7.4 g (56%) of compound *XVc*, m.p. 140 – 142 °C; $[\alpha]_D$ +7.6° (*c* 0.5, dimethylformamide). For $C_{22}H_{20}N_6O_4$ (432.4) calculated: 61.10% C, 4.66% H, 19.44% N; found: 60.91% C, 4.39% H, 19.46% N. ^{1}H NMR spectrum: 3.33 pent, 1 H (ΣJ = 23.4) + 3.54 pent, 1 H (ΣJ = 21.7) (3'-CH_2); 3.92 m, 1 H (ΣJ = 28.3) (2'-CH); 4.15 dd, 1 H (J(1",2') = 7.8, J(gem) = 13.9) and 4.37 dd, 1 H (J(1',2') = 3.7) (1'-CH_2); 4.91 t, (J(3',OH) = 5.7) and 5.21 d, (J(2',OH) = 5.4), 2 × 1 H (OH); 8.31 s, 1 H (H-8); 11.02 and 11.17 2 × s, 2 × 1 H (NH); 7.50 – 7.70 m, 6 H and 7.90 – 8.10 m, 4 H (aromatic H). After exchange: 3.32 dd, 1 H (J(3",2') = 6.5, J(gem) = 11.2) and 3.54 dd, 1 H (J(3',2') = 4.8) (3'-CH_2); 3.92 m, 1 H (J = 22.8) (2'-CH).

2,6-Bis(benzoylamino)-9-(*R*)-(2-hydroxy-3-triphenylmethoxypropyl)purine (*XVIc*)

Trityl chloride (6.0, 19.5 mmol) and 4-dimethylaminopyridine (0.5 g) were added at 70 °C to a stirred mixture of compound *XVc* (6.5 g, 15 mmol), dimethylformamide (40 ml) and pyridine (20 ml). The stirring at 70 °C was continued for 24 h, methanol (5 ml) was added and after 1 h the mixture was concentrated in vacuo to half of the original volume. The residue was diluted with ethyl acetate (500 ml), the solution washed with water (3 × 100 ml), the organic solvent was evaporated, the residue codistilled with toluene (4 × 100 ml) and purified by column chromatography on silica gel (200 ml) in chloroform. Fractions, containing the compound *XVIc* (R_F 0.67 in S1), were combined, the solvent was evaporated and the residue precipitated from ether with light petroleum. Yield 5.0 g (49%) of compound *XVIc*, m.p. 144 – 145 °C; $[\alpha]_D$ +11.8° (*c* 0.5, dimethylformamide). For $C_{41}H_{34}N_6O_4$ (674.7) calculated: 72.98% C, 5.08% H, 12.46% N; found: 73.03% C, 5.11% H, 12.81% N.

9-(*R*)-(3-Hydroxy-2-phosphonomethoxypropyl)-2,6-diaminopurine (*XXVIIc*)

A mixture of compound *XVIc* (4.9 g, 7.25 mmol) and tosyl derivative *XXIII* (3.0 g, 8.6 mmol) was codistilled with dimethylformamide (3 × 20 ml) at 40 °C and 13 Pa. A solution of the residue in dimethylformamide (30 ml) was mixed with 60% dispersion of sodium hydride in paraffin (0.9 g, 22.5 mmol) and the mixture was stirred (calcium chloride protecting tube) at room temperature for 3 days. After addition of 0.1 M methanolic sodium methoxide (200 ml), the mixture was set aside for 2 days at room temperature, neutralized with Dowex 50 X 8 (H^+ form), and made alkaline with triethylamine. The Dowex was filtered off and washed with methanol (200 ml). The combined filtrates were taken down in vacuo and the residue was refluxed with 80% acetic acid (200 ml) for 30 min. After evaporation, the residue was diluted with water (200 ml), the suspension was extracted with ether (3 × 100 ml), the extract was washed with water (50 ml) and the combined aqueous phases were taken down in vacuo. The residue was deionized on a column of Dowex 50 X 8 (H^+ form; 200 ml); the column was washed with 20% aqueous methanol to drop of UV absorption of the eluate and then with 2% ammonia in 20% aqueous methanol. The ammonia UV-absorbing eluate was evaporated to dryness, the residue codistilled with ethanol (3 × 50 ml) and dried in vacuo. The thus-obtained compound *XXVIb* was treated with acetonitrile (40 ml) and bromotrimethylsilane (4 ml), and the obtained solution was allowed to stand overnight at room temperature. The reaction mixture was concentrated, the residue dissolved in water (100 ml) and the solution made alkaline with ammonia and the solvent was again evaporated. The residue was deionized on a column of Dowex 50 X 8 (H^+ form; 150 ml) and the ammonia eluate was concentrated. A mildly alkaline solution of the residue was applied onto a column of Dowex 1 X 2 (acetate form; 150 ml) and the column was washed with water to disappearance of UV absorption. The column was then eluted with a linear gradient 0 – 0.5 M acetic acid (750 ml each). Fraction 0.3 – 0.5 M on evaporation and crystallization from water afforded 1.4 g (60.7%) of compound *XXVIIc*, m.p. 282 – 284 °C; $[\alpha]_D$ +24.8° (*c* 0.5, 0.1 M HCl). For $C_9H_{15}N_6O_5P$ (318.3) calculated: 33.96% C, 4.75% H, 26.41% N, 9.75% P; found: 33.57% C, 4.79% H, 26.15% N, 9.66% P. R_F 0.17 (S7), E_{Up} 0.60. HPLC (S8) k = 0.72.

Further elution with 0.5 M acetic acid afforded (after crystallization from water) 0.55 g (18%) of benzoyl derivative *XXXII*, m.p. 242 – 244 °C. For $C_{16}H_{19}N_6O_6P$ (422.4) calculated: 45.49% C, 4.53% H, 19.90% N, 7.35% P; found: 44.67% C, 4.68% H, 19.93% N, 7.52% P. R_F 0.34 (S7), E_{Up} 0.50. ^{1}H NMR spectrum: 3.45 dd, 1 H (J(3",2') = 5.1, J(gem) = 12.6) + 3.72 dd, 1 H (J(3',2' = 3.7) (3'-CH_2); 3.82 m, 1 H (2'-CH); 4.32 dd, 1 H (J(1",2') = 6.0, J(gem) = 14.8) + 4.39 dd, 1 H (J(1',2') = 4.6) (1'-CH_2); 8.16 s, 1 H (H-8); 3.53 dd, 1 H (J(P–CH) = 9.6, J(gem) = 12.2) + 3.58 dd, 1 H (J(P–CH) = 9.0) (P–CH_2); 7.40 – 7.55 m, 3 H and 7.85 – 7.90 m, 2 H (aromatic H).

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

A) (*R*)-Glycidol (*X*; 4.45 g, 60 mmol) was added at 100 °C to a stirred mixture of cytosine (5.55 g, 50 mmol), cesium carbonate (1 g) and dimethylformamide (100 ml). The stirring at 100 °C was continued for 2 h, the solvent was evaporated, the residue codistilled with toluene (2 × 50 ml), dissolved in 80% methanol (100 ml), and the hot solution was neutralized by addition of Dowex 50 X 8 (H^+ form). The Dowex was filtered off, washed with ethanol (100 ml) and ether was added to turbidity. The product, which crystallized upon standing in a refrigerator, was collected, washed with ethanol and crystallized from 80% aqueous ethanol with addition of ether. Yield 6.1 g (64%) of chromatographically pure compound *XI* (S6, R_F 0.17), m.p. 186 – 188 °C; $[\alpha]_D$ +37.2° (*c* 0.5, dimethylformamide). For $C_7H_{11}N_3O_3$ (185.2) calculated: 45.39% C, 5.99% H, 22.69% N; found: 45.55% C, 6.24% H, 22.84% N.

B) Cesium carbonate (16.3 g, 0.05 mol) was added at 100 °C to a mixture of 4-methoxy-2-pyrimidone (12.6 g, 0.1 mol), 2,3-*O*-isopropylidene-1-*O*-*p*-toluenesulfonyl-D-glycerol (*V*; 34.3 g, 0.12 mol) and dimethylformamide (250 ml) and the mixture was stirred at 100 °C for 12 h under exclusion of moisture. After evaporation in vacuo, the residue was extracted with hot chloroform (3 × 200 ml), the solvent was evaporated and the residue chromatographed on a column of silica gel (400 ml). Crystallization of the product from ethanol–ether afforded 13.2 g (50%) of compound *VI*, identical with the product prepared according to ref.[8]. This product was heated at 110 – 120 °C with saturated methanolic ammonia (300 ml) for 12 h and after cooling the solid was collected, washed with ethanol, ether, and dried. The obtained isopropylidene derivative *IX* was set aside with 0.25 M sulfuric acid (200 ml) overnight, the solution was neutralized with saturated solution of barium hydroxide, heated to 80 °C, filtered through Celite which was then washed with hot water (1 l) and the combined filtrates were taken down in vacuo. Crystallization from 80% aqueous ethanol with addition of ether afforded 7.4 g (40% calculated on compound *VI*) of compound *XIa*, identical with compound obtained by procedure *A*.

Bis(2-propyl) N^4-Benzoyl-1-(*S*)-(2-phosphonomethoxy-3-triphenylmethoxypropyl)cytosine (*XXVIIIa*)

A mixture of trityl derivative[8] *XVIIIa* (24 g, 38 mmol) and tosyl derivative *XXIII* (16.7 g, 48 mmol) was codistilled with dimethylformamide (2 × 100 ml) at 40 °C and 13 Pa, dissolved in dimethylformamide (130 ml), and 60% sodium hydride dispersion in paraffin (4.6 g, 0.114 mol) was added under stirring. The mixture was stirred at room temperature for 3 days (calcium chloride tube). Acetic acid (4.5 ml) was added and the solvent was evaporated at 40 °C and 13 Pa. The residue was dissolved in ethyl acetate (300 ml), the solution washed with water (3 × 100 ml), the aqueous extract extracted with ethyl acetate (2 × 50 ml) and the combined organic phases were dried over magnesium sulfate. After evaporation of the solvent, the residue was chromatographed on silica gel in chloroform, affording 25.0 g (92%) of compound *XXVIIIa* as an amorphous glass. For $C_{40}H_{44}N_3O_7P$ (709.9) calculated: 5.92% N, 4.37% P; found: 6.15% N, 4.55% P.

Bis(2-propyl) 1-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)cytosine (*XXXa*)

A mixture of trityl derivative[8] *XVIIIa* (56.8 g, 90 mmol) and tosyl derivative *XXIII* (39 g, 111 mmol) was codistilled with dimethylformamide (200 ml) at 40 °C and 13 Pa and then dissolved in dimethylformamide (300 ml). The solution was cooled to –10 °C and 60% sodium hydride dispersion in paraffin (10.7 g, i.e., 6.4 g, 0.27 mol of NaH) was added. The mixture was stirred at –10 °C for 1 h, then at 0 °C for 4 h and then at room temperature for 16 h. According to TLC (R_F 0.45 in S5) the reaction was quantitative. The mixture was allowed to stand with 0.1 M methanolic sodium methoxide (1 l) at room temperature overnight and then neutralized by addition of Dowex 50 X 8 (H^+ form). After addition of triethylamine to an alkaline reaction, the mixture was filtered, the Dowex washed with methanol (300 ml), and the filtrate taken down. The residue was codistilled with toluene (3 × 100 ml) and boiled with 80% acetic acid (150 ml) for 40 min. After evaporation, the residue was mixed with water (200 ml) and extracted with ether (2 × 50 ml). The

ethereal extract was washed with water (2 × 25 ml) and the combined aqueous phases were concentrated almost to dryness. The residue was dissolved in 25% methanol (100 ml) and applied onto a column of Dowex 50 X 8 (H^+ form; 250 ml). The column was washed with 25% methanol to drop of the UV absorption, then the Dowex from the column was suspended in 25% methanol (300 ml) and the suspension made alkaline with ammonia to pH 10. The Dowex was filtered, washed with 25% methanol, containing 1% of ammonia, and the combined filtrates were concentrated in vacuo. The residue was codistilled with ethanol (3 × 50 ml) and adsorbed from methanol solution on silica gel (150 ml). This sorbent was layered on a column of silica gel (400 ml) in chloroform and eluted with a gradient of methanol in chloroform. The product (R_F 0.60, S4) was eluted with methanol–chloroform (1 : 9). Fractions containing compound *XXXa* were combined and stirred with dry ether (100 ml). Light petroleum (200 ml) was added and the mixture was left to crystallize at 0 °C. Yield 20.0 g (61%) of compound *XXXa*, m.p. 163 °C; $[\alpha]_D$ −12.9° (*c* 0.5, diemthylformamide). For $C_{14}H_{26}N_3O_6P$ (363.4) calculated: 46.47% C, 7.21% H, 11.57% N, 8.54% P; found: 45.97% C, 7.27% H, 11.45% N, 8.60% P. ^{1}H NMR spectrum: 1.20 + 1.23 2 × d, 2 × 3 H (J(CH$_3$,CH) = 6.4); 1.22 d, 6 H (2 × CH$_3$); 3.42 m, (after exchange 3.40 dd, 1 H (J(3″,2′) = 4.3, J(gem) = 11.9) and 3.45 dd, 1 H (J(3′,2′) = 4.6) (3′-CH$_2$); 3.60 dd, 1 H (J(1″,2′) = 6.7, J(gem) = 12.5) and 3.90 dd, 1 H (J(1′,2′) = 2.75) (1′-CH$_2$); 3.63 m, (2′-CH); 3.73 dd, 1 H (J(P,CH) = 9.5, J(gem) = 14.0) and 3.86 dd, 1 H (J(P,CH) = 8.6) (P–CH$_2$); 4.55 2 × d, 1 H (J(P–OCH) = 7.6) (P–OCH); 4.83 t, 1 H (J(OH,H-3′) = 5.8) (OH); 5.62 d, 1 H (J(5,6) = 7.0) (H-5); 6.98 and 7.07 2 × s, 2 × 1 H (NH$_2$); 7.43 s, 1 H (J(5,6) = 7.0) (H-6).

N^1-Benzoyl-1-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)cytosine (*XXXI*)

Bromotrimethylsilane (2.5 ml) was added to a solution of compound *XXVIIIa* (1.1 g, 1.5 mmol) in acetonitrile (25 ml). After standing overnight under exclusion of moisture, the solvent was evaporated and the residue dissolved in a 5% solution of triethylamine in 80% ethanol (40 ml). After evaporation, the residue was chromatographed on a preparative layer of silica gel in the system S2. The zone of polar compound was eluted with methanol, affording, after evaporation, 0.40 g (69%) of compound *XXXI* as an amorphous foam; R_{Tp} 0.67. For $C_{15}H_{18}N_3O_7P$ (383.4) calculated: 46.99% C, 4.73% H, 10.96% N, 8.10% P; found: 47.46% C, 4.46% H, 10.41% N, 7.86% P. ^{1}H NMR spectrum: 6.01 d, 1 H (J(5,6) = 7.3) (H-5); 7.68, 1 H (H-6); 7.87 m, 2 H and 7.47 m, 3 H (aromatic H); non-resolved multiplets: 3.98 – 4.09 (2 H), 3.69 – 3.88 (3 H), 3.45 – 3.62 (4 H).

1-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)cytosine (*Ia*)

A) From compound *XXXa*. A mixture of compound *XXXa* (18.7 g, 50 mmol), acetonitrile (250 ml) and bromotrimethylsilane (50 ml) was stirred in a stoppered flask to homogeneity and then allowed to stand overnight. According to paper electrophoresis of a hydrolyzed sample, the reaction was quantitative. The solvent was evaporated and the residue codistilled with acetonitrile (50 ml). Water (200 ml) was added and the mixture made alkaline with triethylamine. After 20 min the solvent was evaporated, the residue codistilled with water, applied onto a column of Dowex 50 X 8 (250 ml) and deionized under usual conditions. The ammonia eluate was concentrated and the residue chromatographed on a column of Dowex 1 X 2 (acetate form; 250 ml). The column was first washed with water to drop of UV absorption and the product was eluted with a linear gradient (1 l each) of 0 – 0.2 M acetic acid. Fractions of the main product were combined, the solvent was evaporated and the residue codistilled with water (2 × 50 ml). Crystallization from minimum amount of hot water with addition of 3 volumes of ethanol and ether to turbidity afforded 13.5 g (96%) of 1-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)cytosine (*Ia*), identical with an authentic material.

B) From compound *XXVIIIa*. Bromotrimethylsilane (3 ml) was added to a solution of compound *XXVIIIa* (2.1 g, 3 mmol) in acetonitrile (30 ml). After standing overnight under exclusion of moisture, the solvent was evaporated and the residue dissolved in 5% triethylamine in 80% ethanol (50 ml). The mixture

was again evaporated, the residue was codistilled with ethanol (2 × 50 ml), mixed with 0.05 M methanolic sodium methoxide (100 ml) and allowed to stand overnight at room temperature. The mixture was neutralized with Dowex 50 (H^+ form), made alkaline with triethylamine, the Dowex was filtered off, washed with methanol and the filtrate was evaporated in vacuo. The residue was dissolved in water (100 ml), made alkaline with ammonia and extracted with ether (2 × 25 ml). The aqueous layer was made free from ether, applied onto a column of Dowex 50 (H^+ form; 100 ml) which was then washed with water to disappearance of UV absorption. Elution with dilute (1 : 10) aqueous ammonia afforded a UV-absorbing eluate which, after evaporation, was purified as described for procedure *A*. Yield 0.6 g (71.5%) of 1-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)cytosine (*Ia*), identical with an authentic material.

Bis(2-propyl) N^6-Benzoyl-9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine (*XXXV*)

A mixture of N^6-benzoyl-9-(*S*)-(2-hydroxy-3-triphenylmethoxypropyl)adenine (*XVIIIb*; 13 g, 23.4 mmol) and tosyl derivative *XXIII* (9.15 g, 26 mmol) was evaporated with dimethylformamide (2 × 50 ml) at 40 °C and 13 Pa. The residue was dissolved in dimethylformamide (80 ml), the solution cooled to −20 °C, and 60% suspension of sodium hydride in paraffin (2.8 g, 70 mmol) was added. The mixture was stirred under exclusion of moisture for 3 days, mixed with acetic acid (4.2 ml) and evaporated. The residue was codistilled with toluene (50 ml) and refluxed with 80% acetic acid (150 ml) for 30 min. After evaporation in vacuo, the residue was codistilled with toluene (4 × 50 ml) and dissolved in chloroform (300 ml). The chloroform solution was washed successively with water (50 ml), saturated solution of sodium hydrogen carbonate to neutral reaction, again water, and dried over magnesium sulfate. The solvent was evaporated and the residue chromatographed on a column of silica gel (600 ml) in chloroform and then eluted with chloroform–ethanol (97.5 : 2.5), affording 5.9 g (51%) of compound *XXXV* as an amorphous foam; R_F 0.22 (S2). For $C_{22}H_{30}N_5O_6P$ (491.5) calculated: 14.25% N, 6.31% P; found: 14.27% N, 5.81% P. ^{1}H NMR spectrum: 1.10 d + 1.15 2 × d + 1.19 d (CH_3); 3.79 dd, 1 H (J(P,CH) = 9.4, J(gem) = 14.0) + 3.93 dd, 1 H (J(P,CH) = 8.6) (P–CH_2); 3.54 br t, 2 H (J = 4.5) (3′-CH_2); 3.95 m, 1 H (2′-CH); 4.30 – 4.60 m, 4 H (1′-CH_2 + 2 × P–OCH); 4.99 br t, 1 H (J = 4.5) (OH); 8.72 s, 1 H + 8.40 s, 1 H (H-2 + H-8); 11.14 br, 1 H (NH); 7.47 – 7.73 m, 3 H and 8.00 – 8.43 m, 2 H (aromatic H). After exchange: 3.32 dd, 1 H (J(3″,2′) = 6.5, ΣJ(gem) = 11.2) and 3.54 dd, 1 H (J(3′,2′) = 4.8) (3′-CH_2); 3.92 m, 1 H (ΣJ = 22.8) (2′-CH).

Bis(2-propyl) 9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine (*XXXb*)

A 60% suspension of sodium hydride in paraffin (16.6 g, 0.415 mol) was added at 0 °C to a solution of compound *XVIIIb* (76 g, 0.137 mol) in dimethylformamide (400 ml). The mixture was stirred at this temperature for 1 h, a solution of tosyl derivative *XXIII* (53.5 g, 0.153 mol) in dimethylformamide (80 ml) was added and the stirred mixture was heated at 80 °C for 30 h. After evaporation in vacuo, the residue was mixed with 0.1 M methanolic sodium methoxide (800 ml), set aside at room temperature overnight and neutralized by addition of Dowex 50 X 8 (H^+ form). The suspension was made alkaline with triethylamine, the Dowex was filtered off, washed with methanol (300 ml) and the solvent was evaporated. The residue was refluxed in 80% acetic acid (1 l) for 45 min, the acid was evaporated, and water (1.5 l) was added to the residue. After extraction with ether (4 × 250 ml), the aqueous phase was concentrated to about 200 ml, filtered through Celite which was then washed with water, and the filtrate was applied onto a column of Dowex 50 X 8 (H^+ form; 400 ml). The column was washed with water to drop of UV absorption and bis(2-propyl) 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine (*XXXb*) was eluted with dilute (2.5%) aqueous ammonia. After evaporation in vacuo, the chromatographically practically pure product was codistilled with ethanol (3 × 50 ml) and dried, leaving compound *XXXb* as an amorphous foam (29 g, 55%). This product was used for the preparation of compound *Ib*.

9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine (*Ib*)

Bromotrimethylsilane (40 ml) was added to a solution of compound *XXXb* (29 g, 75 mmol) in acetonitrile (400 ml), the mixture was stirred to homogeneity and then allowed to stand in a stoppered flask at room temperature overnight. The solvent was evaporated, the residue codistilled with acetonitrile (100 ml), and water (200 ml) was added. After 30 min the solution was made alkaline with ammonia, evaporated in vacuo, the residue dissolved in water (200 ml) and deionized on a column of Dowex 50 X 8 (H^+ form; 500 ml). The ammonia UV-absorbing eluate was evaporated and the residue dissolved in water (200 ml), made alkaline with ammonia to pH 9, and applied onto a column of Dowex 1 X 2 (acetate form; 500 ml). The column was washed with 0.05 M acetic acid to drop of UV absorption. As soon as the main portion of the product was eluted, the elution was executed with 0.5 M acetic acid to drop of UV absorption. The combined fractions were evaporated, the residue codistilled with water (3 × 50 ml) and boiled with water (200 ml). Ethanol (600 ml) and acetone (100 ml) were added and the mixture was set aside at room temperature overnight. The product was collected on filter, washed with acetone and ether, and dried in vacuo; yield 19.0 g (79%) of monohydrate of compound *Ib*, m.p. > 300 °C. For $C_9H_{14}N_5O_5P \cdot H_2O$ (321.2) calculated: 33.65% C, 5.02% H, 21.79% N, 9.65% P; found: 33.29% C, 5.39% H, 21.40% N, 9.71% P. $[\alpha]_D$ −25.3° (*c* 0.5, 0.1 M HCl). ^{13}C NMR spectrum (D_2O, NaOD): 152.19 (C-2), 148.79 (C-4), 117.93 (C-5), 155.23 (C-6), 143.21 (C-8), 44.00 s (C-1'), 80.04 d (^3J(P,C) = 11.2) (C-2'), 60.51 s (C-3'), 67.48 d (^1J(P,C) = 152.5) (C–P).

Bis(2-propyl) N^2-Benzoyl-9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)guanine (*XLI*)

A solution of compound *XVIIId* (10.6 g, 18.6 mmol) and tosyl derivative *XXIII* (7.2 g, 20.7 mmol) in dimethylformamide (50 ml) was evaporated to dryness at 40 °C and 13 Pa. The residue was dissolved in dimethylformamide (50 ml), cooled to −20 °C and 60% sodium hydride suspension in paraffin (2.25 g, 56.3 mmol) was added under stirring and exclusion of moisture. The stirring was continued for 30 min at −10 °C and then at room temperature for 24 h. Acetic acid (3.4 ml, 56.3 mmol) was added and the solvent was evaporated at 40 °C and 13 Pa. The residue was codistilled with toluene (2 × 25 ml), 80% acetic acid (120 ml) was added and the stirred mixture was refluxed for 30 min. After evaporation, the residue was coevaporated with toluene (3 × 50 ml) and taken up in chloroform (300 ml). The extract was washed successively (100 ml each) with water, saturated sodium hydrogen carbonate solution and water, and dried over magnesium sulfate. Chromatography on silica gel (200 ml) afforded 7.5 g (80%) of compound *XLI*, m.p. 173 – 174 °C, $[\alpha]_D$ −28.1° (*c* 0.5, dimethylformamide), R_F 0.47 (S2). For $C_{22}H_{30}N_5O_7P$ (507.6) calculated: 52.06% C, 5.96% H, 13.80% N, 6.12% P; found: 52.38% C, 5.82% H, 14.23% N, 5.65% P. ^1H NMR spectrum: 1.135 d + 1.20 d (*J* = 6.2) (CH_3); 3.78 dd, 1 H (*J*(P,CH) = 9.8, *J*(gem) = 14.0) and 3.90 dd, 1 H (*J*(P,CH) = 9.3) (P-CH_2); 3.51 br t, 2 H (*J* = 5.0) (3'-CH_2); 3.84 m, 1 H (2'-CH); 4.21 dd, 1 H (*J*(1'',2') = 7.4, *J*(gem) = 14.0) and 4.34 dd, 1 H (*J*(1',2') = 3.4) (1'-CH_2); 4.50, 2 H (*J*(CH,CH_3) = 6.2) (P-OCH), 4.95 t, 1 H (*J*(3',OH) = 5.3) (OH); 7.96 s, 1 H (H-8); 11.96 br s + 12.27 br s, 2 × 1 H (NH); 7.50 – 7.70 m, 3 H and 7.90 – 8.10 m, 2 H (aromatic H).

9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)guanine (*Id*)

A solution of compound *XVIIId* (20.0 g, 35 mmol) in dimethylformamide (100 ml) was mixed at 0 °C with 60% sodium hydride suspension in paraffin (4.20 g, 105 mmol). After stirring for 1 h at 0 °C, a solution of tosyl derivative *XXIII* (13.5 g, 38.5 mmol) in dimethylformamide (20 ml) was added dropwise and the mixture was stirred at 50 °C for 24 h. After evaporation at 40 °C and 13 Pa, the residue was mixed with 0.1 M methanolic sodium methoxide (200 ml) and set aside overnight. The mixture was neutralized with Dowex 50 X 8 (H^+ form), made alkaline with triethylamine, filtered and the Dowex washed with methanol (200 ml). The filtrate was taken down in vacuo and refluxed with 80% acetic acid (250 ml) for 45 min under stirring. After evaporation, water (400 ml) was added and the solution extracted with ether

(2 × 100 ml). The aqueous phase was concentrated to about 100 ml and deionized on a column of Dowex 50 X 8 (H^+ form; 200 ml). The ammonia eluate was evaporated, the residue codistilled with ethanol (2 × 50 ml), dried and the obtained compound *XXXd* (9.4 g, 66.5%) was stirred with a mixture of acetonitrile (200 ml) and bromotrimethylsilane (20 ml) to homogeneity. The mixture was then allowed to stand at room temperature overnight, the solvent was evaporated and the residue codistilled with acetonitrile (50 ml). Water (100 ml) was added and after standing for 30 min the mixture was made alkaline with ammonia and water was evaporated in vacuo. The residue was deionized on a column of Dowex 50 X 8 (H^+ form; 250 ml) and the ammonia eluate evaporated. The residue was dissolved in water (100 ml), made alkaline to pH 9 with ammonia, and applied onto a column of Dowex 1 X 2 (acetate form; 200 ml). The column was washed with 0.05 M acetic acid to drop of UV absorption of the eluate and then the product was eluted with 2 M acetic acid. The UV-absorbing eluate of the product was evaporated, the residue codistilled with water (3 × 50 ml) and crystallized from water to give 6.30 g (80% calculated on compound *XXXd*) of monohydrate of compound *Id*, m.p. 198 °C, identical with an authentic sample; $[\alpha]_D$ −51.0° (*c* 0.5, 0.1 M HCl). For $C_9H_{14}N_5O_6P \cdot H_2O$ (337.4) calculated: 32.04% C, 4.78% H, 20.76% N, 9.20% P; found: 32.37% C, 4.58% H, 20.74% N, 9.52% P. ^1H NMR spectrum (D_2O + NaOD): 3.54 d, 2 H (J(P,CH) = 9.5) (P–CH_2); 3.55 dd, 1 H (J(3",2') = 6.0, J(gem) = 12.0) + 3.77 dd, 1 H (J(3',2') = 3.5) (3'-CH_2); 3.80 m, 1 H (2'-CH); 4.18 2 × dd (J(1',2') = 4.5, J(1",2') = 6.0, J(gem) = 14.0) (1'-CH); 7.82 s, 1 H (H-8).

9-(*S*)-(2,3-Diphosphonomethoxypropyl)adenine (*XLIII*)

A solution of compound *XXXV* (3.1 g, 6.3 mmol) and tosyl derivative *XXIII* (2.7 g, 7.7 mmol) in dimethylformamide (50 ml) was evaporated at 40 °C and 13 Pa. The residue was dissolved in dimethylformamide (25 ml), cooled to −20 °C, and 60% sodium hydride suspension in paraffin (0.80 g, 20 mmol) was added. After stirring for 48 h under exclusion of moisture, methanol (120 ml) was added, the mixture was set aside overnight and neutralized with Dowex 50 X 8 (H^+ form). The mixture was made alkaline with triethylamine, the Dowex filtered off, the solvent evaporated, the residue codistilled with toluene (3 × 25 ml) and deionized on a column od Dowex 50 X 8 (H^+ form; 200 ml; elution with 20% aqueous methanol to drop of UV absorption, then with 2.5% ammonia in 20% methanol). The ammonia eluate was taken down, the residue was dried in vacuo and allowed to stand overnight with bromotrimethylsilane (10 ml) in acetonitrile (100 ml) under exclusion of moisture. After evaporation, addition of water (100 ml) and alkalization with ammonia, the solvent was again evaporated and the residue applied onto a column of Dowex 50 X 8 (H^+ form; 200 ml). Water eluted (with retention) the product which, after evaporation, was codistilled with ethanol, mixed with ethanol, collected, washed with ether and dried in vacuo. Yield 0.90 g (36%) of compound *XLIII*, not melting up to 300 °C. For $C_{10}H_{17}N_5O_8P_2$ (397.4) calculated: 17.63% N, 15.62% P; found: 17.74% N, 16.02% P. R_{Up} 1.22 (*Ib*, 0.70). HPLC: k = 3.56 (S9).

Reaction of Compound *XVIIb* with Excess of Tosyl Derivative *XXIII*

A solution of compound *XVIIb* (1.57 g, 5 mmol) and tosyl derivative *XXIII* (4.2 g, 12 mmol) in dimethylformamide (50 ml) was cooled to −20 °C, 60% sodium hydride dispersion in paraffin (1.44 g, 36 mmol) was added and the mixture was stirred at room temperature for 3 days under exclusion of moisture. Methanol (100 ml) was added and, after standing overnight, the mixture was neutralized with Dowex 50 X 8 (H^+ form), made alkaline with triethylamine, filtered, and the filtrate was taken down. The residue was codistilled with toluene (3 × 25 ml) and deionized on a column of Dowex 50 X 8 (H^+ form; 100 ml; elution with 20% aqueous methanol to drop of UV absorption, then with 2.5% ammonia in 20% methanol), the ammonia eluate was taken down and dried in vacuo. The residue was allowed to stand overnight with acetonitrile (50 ml) and bromotrimethylsilane (5 ml) under exclusion of moisture. After evaporation, water (100 ml) was added, the mixture was made alkaline with ammonia and the water was evaporated.

According to paper electrophoresis, the mixture contained exclusively compounds of E_{Up} 0.70, corresponding to compound *Ib*, and no compound *XLIII* (E_{Up} 1.22).

9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine 3'-Phosphate (*XXXVII*)

Diethyl chlorophosphate (0.70 ml, 830 mg, 4.8 mmol) and bis(2-propyl)ethylamine (0.70 ml, 529 mg, 4.1 mmol) were added in succession to a solution of compound *XXXV* (1.6 g, 3.2 mmol) in dichloromethane (30 ml). The mixture was stirred for 4 h, both reagents (0.35 ml each) were again added and the stirring was continued overnight under exclusion of moisture. The solvent was evaporated and the residue was dissolved in 0.2 M methanolic sodium methoxide (50 ml) and allowed to stand overnight under exclusion of moisture. The mixture was neutralized by addition of Dowex 50 X 8 (H^+ form) and made alkaline with triethylamine. The solvent was evaporated, the residue deionized on a column of the same ion-exchanger (100 ml) and the ammonia eluate was taken down in vacuo. The residue was codistilled with ethanol (2 × 25 ml), dried, and treated with acetonitrile (40 ml) and bromotrimethylsilane (4 ml). After standing overnight and evaporation in vacuo, the residue was dissolved in water (50 ml), the solution made alkaline with ammonia and taken down. The residue was applied onto a column of Dowex 50 X 8 (100 ml) and the column was washed with water. After washing out the salts, the product was eluted with retention. Evaporation and crystallization from water (with addition of 3 volumes of ethanol) afforded 0.60 g (49%) of compound *XXXVII*, not melting up to 300 °C; E_{Up} 1.28 (*Ib* 0.75). For $C_9H_{15}N_5O_8P_2$ (383.3) calculated: 28.20% C, 3.32% H, 15.38% N, 13.63% P; found: 28.35% C, 3.10% H, 15.25% N, 13.47% P. The product was homogeneous according to HPLC (k = 0.60 in S8, for *Ib* k = 1.71).

9-(*R*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine 3'-Phosphate (*XL*)

The title compound was prepared from compound *XXXVIII* (1.15 g, 2.3 mmol) in dichloromethane (50 ml) and diethyl chlorophosphate (2 ml total) and bis(2-propyl)ethylamine (2 ml total), analogously as described for compound *XXXVII*. The reaction time was 48 h and the work-up was the same as for compound *XXXVII*. Yield 46% of compound *XL*, homogeneous according to HPLC (k = 0.60 in S8, for *Ib* k = 1.71): E_{Up} = 1.28 (for *Ib* 0.75).

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