GENERAL METHOD OF PREPARATION OF N-[(*S*)-(3-HYDROXY-2-PHOSPHONOMETHOXYPROPYL)] DERIVATIVES OF HETEROCYCLIC BASES

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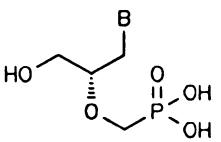
Reaction of (*R*)-1-O-*p*-toluenesulfonyl-1,2,3-propanetriol (*IV*) with N-trimethylacetylimidazole (*II*) afforded (*R*)-1-O-*p*-toluenesulfonyl-3-O-trimethylacetyl-1,2,3-propanetriol (*V*) which was reacted with dimethoxymethane in the presence of phosphorus pentoxide to give (*R*)-2-O-methoxymethyl-1-O-*p*-toluenesulfonyl-3-O-trimethylacetyl-1,2,3-propanetriol (*VI*). Compound *VI* was treated with acetic anhydride and boron trifluoride etherate and the obtained 2-acetoxy derivative *VII* reacted with bromotrimethylsilane to give the intermediary bromomethyl ether *VIII*. Compound *VIII* on reaction with tris(2-propyl) phosphite afforded (*R*)-2-O-bis(2-propyl)phosphonomethyl-1-O-*p*-toluenesulfonyl-3-O-trimethylacetyl-1,2,3-propanetriol (*IX*). Condensation of synthon *IX* with sodium salts of adenine, 2,6-diaminopurine, or with cytosine, 6-azacytosine or 2-chloroadenine in the presence of cesium carbonate, afforded fully protected diesters *X* and *XIIIb* which on methanolysis and reaction with bromotrimethylsilane gave N-[(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)] derivatives of adenine (*XIa*), 2-chloroadenine (*XIb*), 2,6-diaminopurine (*XIc*), cytosine (*XIVa*) and 6-azacytosine (*XIVb*). In an analogous reaction, sodium salt of 4-methoxy-2-pyrimidone reacted with compound *IX* to give an intermediate *XIIIa* which on treatment with methanolic ammonia and subsequent deblocking under the same conditions also afforded the cytosine derivative *XIVa*. Sodium salt of 2-amino-6-chloropurine was in this way converted into the corresponding 2-aminopurine derivative *XVIII*. Deprotection of this compound gave 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)-2-aminopurine (*XIX*).

(*S*)-N-(3-Hydroxy-2-phosphonomethoxypropyl) derivatives of purine and pyrimidine bases *I* (HPMP-derivatives) are of great importance as agents of significant efficacy against DNA viruses which cause many serious diseases of man and animals. They exhibit a significant effect against herpesviruses, adenoviruses and poxviruses and their effect is not dependent on phosphorylation with virus thymidine kinase[1,2]. So far, HPMP-derivatives were prepared from (*S*)-N-(2,3-dihydroxypropyl) derivatives of purine and pyrimidine bases, particularly by reaction of their 3'-O-chloromethylphosphonyl esters with aqueous solutions of alkali metal hydroxides[3,4], from N-benzoyl-3'-O-trityl or N-dimethylaminomethylene-3'-O-trityl derivatives[4 – 6], or N,3'-O-ditrityl derivatives by reaction with dialkyl *p*-toluenesulfonyloxymethanephosphonates or methanesulfonyloxymethanephosphonates[7,8] in the presence of sodium hydride, and subsequent removal of the protecting groups by acid or alkaline hydrolysis and finally with bromotrimethylsilane. All these methods require preparation of the

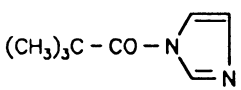
optically active (*S*)-N-(2,3-dihydroxypropyl) derivative[9] and, contingently, its multistep protection prior to the synthesis proper. It is obvious that such procedure is not very practical in cases, requiring preparation of HPMP-derivatives with modified heterocyclic base; also the effectivity of preparing HPMP-derivatives on a large scale suffers from the fact that the first step of a multistep reaction sequence is just the synthesis of optically active compound. These facts led us to the idea of a reversed strategy, i.e., a route, consisting in condensation of the heterocyclic base (its silyl derivative or alkali metal salt) with suitable optically active organophosphorus synthon containing a preformed structure of the side-chain in the desired HPMP-derivative.

Such approach has already been utilized[10], however, its essential drawback is that the hydroxyl of the synthon is protected with the benzyl group which, though stable during the preparation and condensation of the synthon, has to be removed in the final stage by hydrogenation. Such a process is not suitable for those heterocyclic bases which are easily hydrogenated (derivatives of cytosine, 5-alkyluracils, etc.) or reduced (bases containing C-halogen bonds, azido groups, etc.); at least, it lowers the reaction yield and complicates the isolation of pure product. Therefore, we decided to elaborate a synthesis of another type of chiral synthon which could be used in both enantiomeric series and which would not contain protecting groups other than those removable by methanolysis.

A suitable organophosphorus synthon for the preparation of HPMP-derivatives of the general formula *I* requires (i) an appropriate reactive group capable of substitution reaction with an activated form of the heterocyclic base, (ii) suitable groups protecting the primary hydroxyl functionality of the desired HPMP-derivatives in position 3′ which could be regiospecifically introduced and again removed after the reaction without destruction of the product, and (iii) suitable ester protecting groups on the phosphonic acid moiety (as obvious from the previous studies, the phosphonate group must be protected as a diester). The whole synthetic sequence leading to the chiral synthon should start from an easily accessible chiral compound with preservation of optical purity of the synthon as well as the final product.



I



II

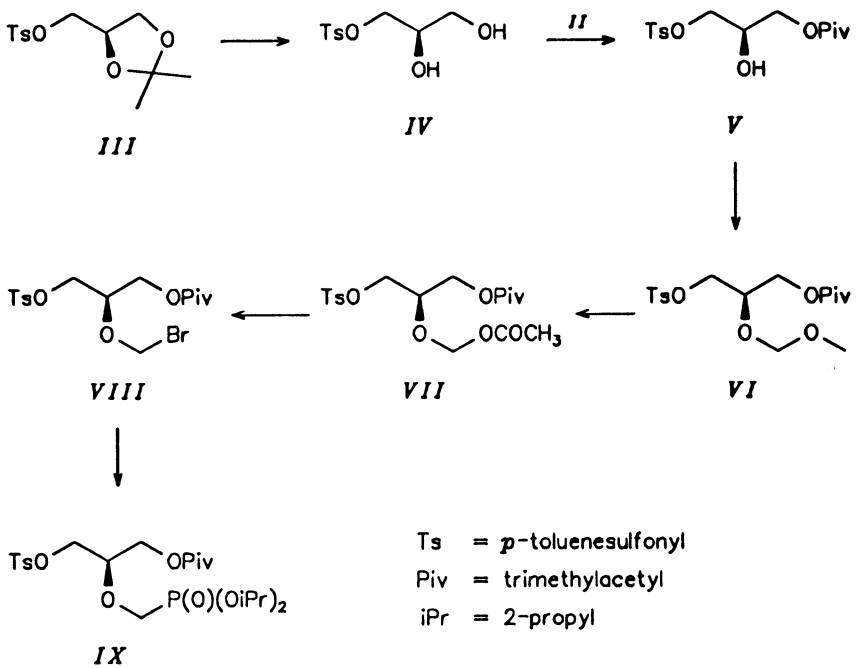
As shown by previous experiments, the required N-substitution at the heterocyclic bases can be well realized with chlorine atom or *p*-toluenesulfonyloxy or methanesulfonyloxy groups as the reactive groups in the synthon. We have chosen the *p*-toluenesulfonyloxy derivatives which are easily accessible from alcohols and can be well purified. As the starting compound for the preparation of the synthon we used (*R*)-3-O-*p*-toluene-

sulfonyloxy-1,2,3-propanetriol (*IV*), prepared from (*R*)-4-O-*p*-toluenesulfonylmethyl-2,2-dimethyl-1,3-dioxolane (*III*, ref.[11]) by acid hydrolysis in the presence of a cation-exchanging resin. The primary hydroxyl was protected by trimethylacetyl group which could be removed by methanolysis. The selective trimethylacetylation of compound *IV* at the primary hydroxyl was not simple: under standard conditions in pyridine or an inert solvent in the presence of a tertiary base the reaction afforded a mixture of mono- and bispivaloyl derivatives. Selective reaction was achieved only by reaction with an equimolecular amount of N-trimethylacetylimidazole (*II*), obtained by reaction of trimethylacetyl chloride with imidazole in chloroform. This product was stable so far as to withstand extraction of salts from chloroform solution with water.

The reaction of compound *IV* with the imidazole *II* in methylene chloride in the presence of triethylamine at room temperature proceeded relatively slowly, but selectively, at the primary hydroxyl to give the product *V*. The 1,2-disubstituted derivative, arising in small amount (< 3%), was easily removed by chromatography on silica gel.

For attachment of the phosphonomethyl ether functionality to the hydroxyl group in position 2 of compound *V* we investigated various methods. Without positive result, we tried transformation of the hydroxyl functionality via 2-methylthiomethyl ethers and their activation with halogen[12], published recently for an analogous case[13].

The method of choice appeared to be the alternative used for preparation of bromomethyl ethers: alcohol *V* was first converted to methoxymethyl ether *VI* (Scheme 1);



SCHEME 1

although reaction with methyl chloromethyl ether in the presence of a base could undoubtedly be used for this purpose, for obvious reasons we prefered transacetalization of dimethoxymethane in the presence of phosphorus pentoxide.

The methoxymethyl group in compound *VI* was converted into acetoxymethyl derivative *VII* by reaction with acetic anhydride in the presence of a Lewis acid, e.g. boron trifluoride etherate. The compound *VII* was immediately treated with bromotrimethylsilane to give the reactive 2-bromomethyl ether *VIII*. Even at relatively high temperature, this reaction proceeded slowly and its course had to be followed by thin-layer chromatography. The crude product *VIII* was then reacted with trialkyl phosphite. The ester of choice appeared to be tris(2-propyl) phosphite: the obtained bis(2-propyl) phosphonate in the final synthon *IX* cannot alkylate the heterocyclic base as observed e.g. in the case of the analogous ethyl or particularly methyl esters. The reaction with tris(2-propyl) phosphite proceeded smoothly and the final product *IX* was obtained by chromatography on silica gel.

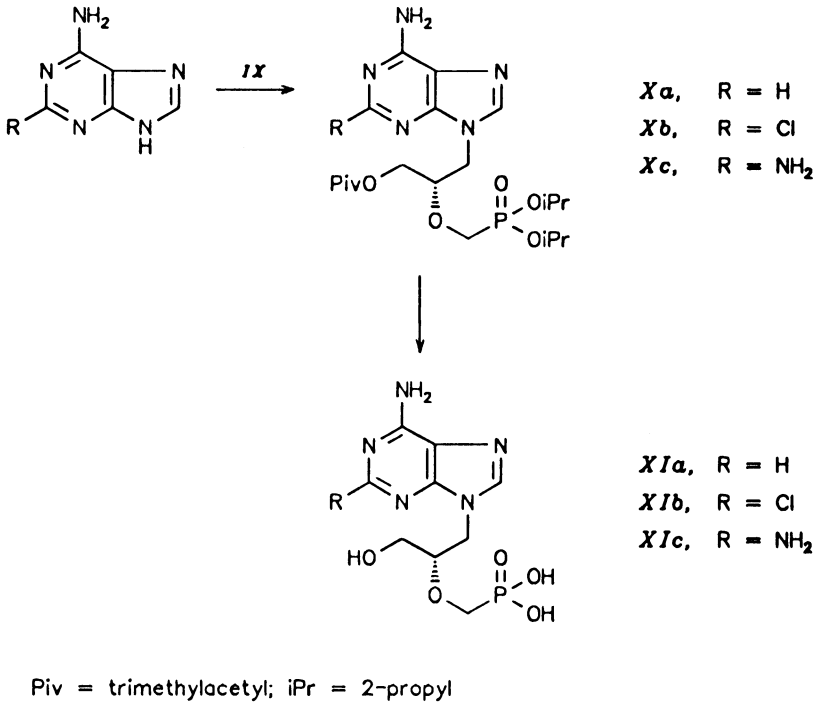
Obviously, an analogous reaction pathway could be realized also with derivatives of the (*S*)-glycero series. The (*S*)-enantiomer of the tosyl derivative *IV* is easily accessible e.g. from D-ribonolactone[14].

Condensation of the synthon *IX* with heterocyclic bases was studied from the viewpoint of the structure and optical purity of this compound and the phosphonates *I*, as well as of the suitability for the desired purpose. The first aspect was checked by the synthesis of known derivatives of adenine, 2,6-diaminopurine and cytosine.

The reaction of adenine and 2,6-diaminopurine was carried out with the sodium salts generated by treatment with sodium hydride in dimethylformamide. The intermediary fully protected diesters *Xa* and *Xc* were methanolyzed without isolation and, after deionization, the ester bond was cleaved with bromotrimethylsilane. The arising phosphono derivative *XIa* was isolated by chromatography on an ion-exchanger whereas compound *XIc* was directly liberated from the salt by acidification (Scheme 2). The purity and identity with authentic compounds[4,5] was proved by HPLC analysis; the values of optical rotation also corresponded to the published[5] data. The optical purity of thus-prepared compounds *XI*, as proved by HPLC in 4 mM $CuSO_4$ and 4 mM L-phenylalanine at pH 3.1, was higher than 95%.

Because of its exceptional biological effects, the cytosine derivative *XIVa* (HPMPC) belongs to the most interesting compounds of this series and is also the first HPMP-derivative used in clinical trials. We have proved the applicability of the synthon *IX* to the preparation of this compound in two ways. Reaction of sodium salt of 4-methoxy-2-pyrimidone (*XII*) with synthon *IX* gave first the fully protected intermediate *XIIIa* which was ammonolyzed to give the cytosine derivative *XIIIb*. The same compound was also obtained directly by alkylation of cytosine *XVa* with the synthon *IX*: in this case we successfully made use of the effect of cesium carbonate, employed in the condensation instead of sodium salt of cytosine[5,15]. The yield of (*S*)-HPMPC (*XIVa*),

obtained by deblocking of intermediate *XIIIb*, is higher in the latter pathway than in the synthesis starting from the base *XII*.



Xa, R = H
Xb, R = Cl
Xc, R = NH_2

XIa, R = H
XIb, R = Cl
XIc, R = NH_2

Piv = trimethylacetyl; iPr = 2-propyl

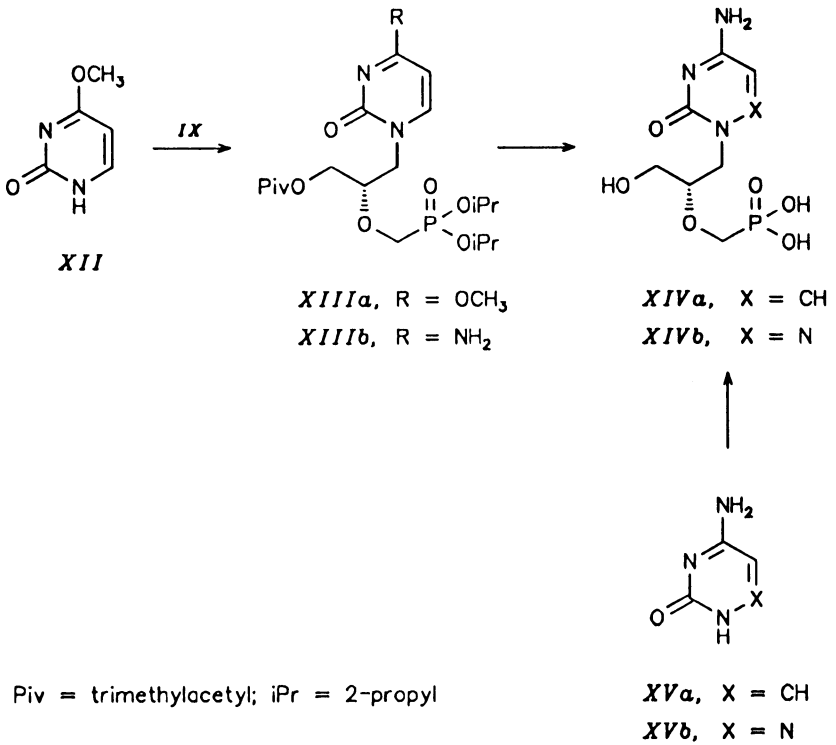
SCHEME 2

Analogously, also 6-azacytosine (*XVb*) reacted with the synthon *IX* in the presence of cesium carbonate and subsequent deblocking gave the hitherto undescribed 6-aza analog of HPMPC, compound *XIVb* (Scheme 3).

The suitability of the synthon *IX* for the preparation of HPMP-derivatives derived from not easily accessible heterocyclic bases is illustrated by synthesis of 2-chloro derivative of HPMPA, compound *XIb*. This derivative was prepared by reaction of 2-chloroadenine with synthon *IX* in the presence of cesium carbonate. The fully protected intermediate *Xb* was isolated in high yield and subjected to methanolysis of the trimethylacetyl functionality and then to reaction with bromotrimethylsilane to remove the ester moieties. Analogously to the 2,6-diaminopurine derivative *XIc*, the phosphonate *XIb* was obtained by acidification of a solution of its ammonium salt, thanks to insolubility of its zwitter-ion form in water.

The synthon *IX* was also used in the preparation of 1- and 3-deazaadenine analogs of compound *XIa*; these syntheses will be described elsewhere[8]. Its use may also be

advantageous in cases where conditions required for attaching phosphonomethyl ether functionality to a specifically protected 2,3-dihydroxypropyl derivative (particularly generation of alkoxide anion by treatment with sodium hydride) are not permissible for



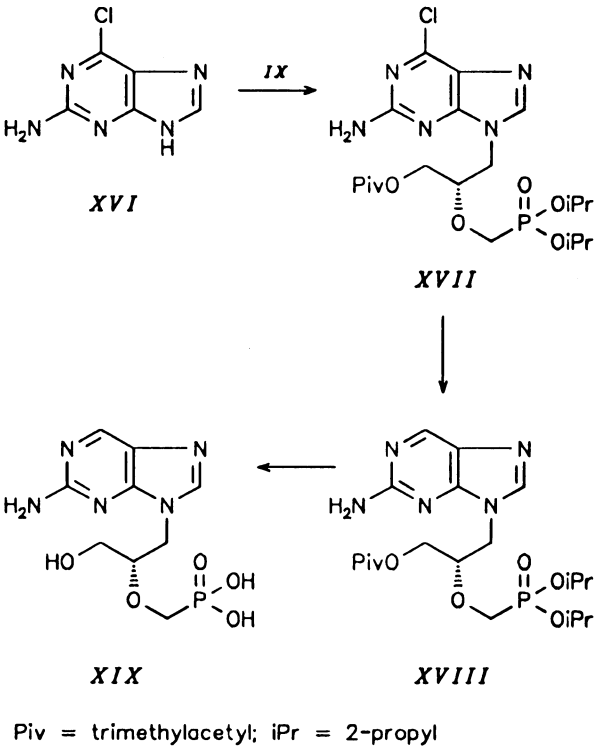
SCHEME 3

the heterocyclic base. Such a case is illustrated by the synthesis of 2-amino-9-[(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)]purine (*XIX*) (Scheme 4): sodium salt of 2-amino-6-chloropurine (*XVI*) on alkylation with synthon *IX* affords the fully protected 6-chloro derivative *XVII*; this intermediate may be used for further transformations of the C–Cl bond which in the said reaction is not destructed. Its hydrogenolysis leading to an analogous fully protected 2-aminopurine derivative *XVIII* may serve as an example. Deblocking under the usual conditions afforded phosphonate *XIX*, identical with an authentic sample[4].

The mentioned examples document the advantage of using the synthon of the type *IX* under certain conditions in the preparation of HPMP-derivatives *I*. The simplicity of performance, as well as the use of methanolysis and reaction with bromotrimethylsilane as the only deprotection steps (with exclusion of hydrogenation), predetermine this

reaction also for the use on the ultramicro scale, e.g. for the preparation of HPMP-derivatives with an isotopically labelled base.

The biological activity of most HPMP derivatives prepared in this paper (*XIa*, *XIc*, *XIVa* and *XIX*) has already been described[16]. Of the hitherto undescribed compounds, derivatives *Xb* and *XIVb* showed no significant in vitro effect against the DNA viruses tested (HSV-1, HSV-2, vaccinia, CMV) or retroviruses (HIV-1, HIV-2)[17].



SCHEME 4

EXPERIMENTAL

Methods. 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 measured 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 or Varian Unity 500 instruments in deuteriochloroform (unless stated otherwise) with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. Thin-layer chromatography was performed on Silufol

UV_{254}, column chromatography on Silpearl silica gel (both Kavalier, Votice, The Czech Republic). Solvent systems for TLC: S1 chloroform, S2 chloroform–methanol (95 : 5), S3 chloroform–methanol (4 : 1). High performance liquid chromatography was carried out on 250 × 4 mm or 250 × 17 mm columns packed with Separon SGiX C18 (5 μm or 10 μm; Laboratorní přístroje, Prague, The 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. Dimethoxymethane, bromotrimethylsilane, pivaloyl chloride, sodium hydride, cesium carbonate, tris(2-propyl) phosphite, acetonitrile, and dimethylformamide were Janssen (Belgium) products, 2-amino-6-chloropurine was purchased from Mack Co. (Germany). Adenine, cytosine, palladium on carbon, 2,6-dichloropurine, and imidazole were obtained from Fluka (Switzerland), 2,6-diaminopurine was a Tokyo Kasei (Japan) product. 4-Methoxy-2-pyrimidone was prepared according to a published procedure[18]. 2-Chloroadenine was obtained by heating 2,6-dichloropurine with methanolic ammonia at 110 °C (ref.[19]). Dimethylformamide, dichloromethane and acetonitrile were dried by distillation from phosphorus pentoxide and stored over molecular sieves. (*R*)-4-*p*-Toluenesulfonyloxymethyl-2,2-dimethyl-1,3-dioxolane was prepared according to ref.[11].

(*R*)-3-O-*p*-Toluenesulfonyl-1,2,3-propanetriol (*IV*)

A mixture of (*R*)-4-*p*-toluenesulfonyloxymethyl-2,2-dimethyl-1,3-dioxolane (50 g, 0.175 mol), Dowex 50X8 (H^+ form, 10 g) and methanol (200 ml) was stirred at 60 °C for 7 h, the solvent was evaporated, the residue mixed with chloroform and filtered through a column of silica gel (100 ml). The filtrate was dried over magnesium sulfate and after filtration taken down at 40 °C and 2 kPa, affording 41.4 g (96%) of sirupy (*R*)-3-O-*p*-toluenesulfonyl-1,2,3-propanetriol (*IV*), completely pure according to chromatography in S2; $[\alpha]_D$ −10.0° (*c* 5.0, methanol).

(*R*)-3-O-*p*-Toluenesulfonyl-1-trimethylacetyl-1,2,3-propanetriol (*V*)

Imidazole (27.2 g, 0.4 mol) was added to a solution of trimethylacetyl chloride (24.2 g, 0.2 mol) in chloroform (400 ml) and the mixture was stirred at room temperature for 2 h. The solution was extracted with ice-cold water (3 × 100 ml), dried over magnesium sulfate and the solvent evaporated. Yield 28.2 g (93%) of N-trimethylacetylimidazole (*II*) which was used in the next step.

Triethylamine (20 ml) was added to a solution of (*R*)-3-O-*p*-toluenesulfonyl-1,2,3-propanetriol (41.4 g, 168 mmol) and N-trimethylacetylimidazole (25.6 g, 168 mmol) in dry dichloromethane (250 ml). The mixture was set aside at room temperature for 7 days (until the starting compound disappeared, as followed by TLC on silica gel in S1; R_F 0.45). Methanol (10 ml) was added, and the mixture was taken down. The residue was dissolved in chloroform (50 ml) and filtered through a column of silica gel (400 ml). After evaporation, the obtained product was dried in vacuo; yield 48.3 g (88%) of (*R*)-3-O-*p*-toluenesulfonyl-1-O-trimethylacetyl-1,2,3-propanetriol (*V*); $[\alpha]_D$ −2.0° (*c* 0.5, chloroform). For $C_{15}H_{22}O_6S$ (330.4) calculated: 54.53% C, 6.71% H, 9.70% S; found: 54.16% C, 6.44% H, 9.43% S. ^1H NMR spectrum: 1.17 s, 9 H (CH_3, Piv); 2.46 s, 3 H (CH_3 (Ts)); 4.00 – 4.28 m, 5 H (H-1, H-2, H-3); 5.50 b, 1 H (OH); 7.36 d + 7.80 d, 2 + 2 H (aromatic H, J = 8.3).

(*R*)-2-O-Methoxymethyl-3-O-*p*-toluenesulfonyl-1-O-trimethylacetyl-1,2,3-propanetriol (*VI*)

Dimethoxymethane (25 ml), followed by phosphorus oxychloride (13 g, 90 mmol), was added to a stirred solution of compound *V* (48 g, 146 mmol) in dichloromethane (300 ml). After stirring for 30 min, the suspension was filtered through Celite which was then washed with chloroform (200 ml). The filtrate was taken down, the residue was dissolved in chloroform (50 ml) and passed through a column of silica gel

(200 ml) in chloroform. Compound *VI* (48.1 g, 88%) was obtained as a colourless oil, $[\alpha]_D$ −2.0° (*c* 0.58, chloroform), R_F 0.70 (S2). For $C_{17}H_{26}O_7S$ (374.4) calculated: 54.53% C, 7.00% H, 8.56% S; found: 54.76% C, 7.23% H, 8.79% S. ^{1}H NMR spectrum: 1.15 s, 9 H (CH_3 (Piv)); 2.45 bs, 3 H (CH_3 (Ts)); 3.33 s, 3 H (OCH_3); 3.95 – 4.24 m, 5 H (H-1, H-2, H-3); 4.63 s, 2 H (OCH_2O); 7.36 d + 7.80 d, 2 + 2 H (aromatic H, J = 8.3).

(*R*)-2-*O*-Acetoxymethyl-3-*O*-*p*-toluenesulfonyl-1-*O*-trimethylacetyl-1,2,3-propanetriol (*VII*)

Boron trifluoride etherate (4.4 ml) was added at 0 °C to a mixture of compound *VI* (48 g, 128 mmol) and acetic anhydride (17 ml). The mixture was stirred at 0 °C for 2 h, poured into a suspension of sodium hydrogen carbonate in ice-cold water (20 g in 100 ml) and extracted with ether (3 × 30 ml). The combined extracts were washed with saturated solution of sodium hydrogen carbonate (20 ml) and with water (20 ml), dried over magnesium sulfate, filtered and the solvent was evaporated to give compound *VII* (49.7 g, 96%) as a colourless oil, $[\alpha]_D$ −3.0° (*c* 0.47, chloroform). For $C_{18}H_{26}O_8S$ (402.5) calculated: 53.72% C, 6.51% H, 7.97% S; found: 53.43% C, 6.33% H, 7.71% S. ^{1}H NMR spectrum: 1.16 s, 9 H (CH_3 (Piv)); 2.09 s, 3 H (CH_3 (Ac)); 2.46 bs, 3 H (CH_3 (Ts)); 4.00 – 4.26 m, 5 H (H-1, H-2, H-3); 5.25 s, 2 H (OCH_2O); 7.36 d + 7.80 d, 2 + 2 H (aromatic H, J = 8.3).

(*R*)-2-*O*-Bis(2-propyl)phosphonomethyl-1-*O*-*p*-toluenesulfonyl-3-*O*-trimethylacetyl-1,2,3-propanetriol (*IX*)

A mixture of compound *VII* (as obtained in the preceding experiment; 49 g, 122 mmol), toluene (100 ml) and bromotrimethylsilane (28.4 ml) was refluxed under argon (calcium chloride protecting tube) until the starting compound disappeared (TLC in S2) (48 h). The mixture was cooled to 40 °C, the volatile parts were evaporated and the residue was codistilled with toluene (50 ml) under argon. Tris(2-propyl) phosphite (30.8 ml) was added and the stirred mixture was heated at 100 °C for 2 h, the reaction being monitored by TLC in S1. After evaporation in vacuo, the residue was codistilled with toluene (50 ml), dissolved in chloroform (50 ml), filtered through a column of silica gel (200 ml) and eluted with a gradient of ethyl acetate (up to 20%) in chloroform. Evaporation of the solvent and drying afforded (*R*)-2-*O*-bis(2-propyl)phosphonomethyl-1-*O*-*p*-toluenesulfonyl-3-*O*-trimethylacetyl-1,2,3-propanetriol (*IX*) (52 g, 83%), $[\alpha]_D$ −0.5° (*c* 0.40, chloroform). For $C_{22}H_{37}O_9PS$ (508.6) calculated: 51.96% C, 7.33% H, 6.09% P, 6.30% S; found: 53.02% C, 7.39% H, 6.20% P, 6.21% S. ^{1}H NMR spectrum: 1.21 s, 9 H (CH_3 (Piv)); 1.31 d, 12 H (CH_3 (iPr), J = 6.4); 2.45 bs, 3 H (CH_3 (Ts)); 3.77 – 4.74 m, 9 H (H-1, H-2, H-3 + CH_2P + CH (iPr)); 7.36 d + 7.80 d, 2 + 2 H (aromatic H, J = 8.3).

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

A mixture of adenine (0.27 g, 2 mmol), sodium hydride (48 mg, 2 mmol) and dimethylformamide (5 ml) was heated at 80 °C for 1 h. A solution of compound *IX* (1.16 g, 2 mmol) in dimethylformamide (5 ml) was added and the mixture was heated at 100 °C for 50 h. After evaporation, the residue was purified by chromatography on silica gel in chloroform with increasing concentration of methanol (final concentration 5%). The obtained product (R_F 0.80; S3) was dissolved in methanol (5 ml) and stirred with 0.1 M methanolic sodium methoxide (2 ml) for 5 h. The mixture was neutralized with Dowex 50X8 (H^+ form) and the suspension applied onto a column of the same ion-exchanger (20 ml). The column was washed with water until the UV absorption at 254 nm dropped to the original value and then with dilute (1 : 10) aqueous ammonia. The UV-absorbing eluate was evaporated and dried in vacuo. The residue was mixed with acetonitrile (8 ml) and bromotrimethylsilane (0.7 ml) and the mixture was set aside at room temperature for 24 h. The solvent was evaporated, the residue codistilled with acetonitrile (5 ml) and mixed with water (20 ml) and triethylamine (2 ml). After standing for 30 min, the solvents were again evaporated, the residue was dissolved in water (10 ml) and deionized on Dowex 50 as described above. The obtained residue was

dissolved in water (10 ml), made alkaline (pH 9 – 10) with ammonia and applied onto a column of Dowex 1X2 (acetate form; 20 ml). After washing with water until the UV absorption of the eluate at 254 nm dropped to the original value, the product was eluted with a linear gradient of acetic acid (to 0.5 mol l^{-1}, total volume 500 ml). The main UV-absorbing fraction was evaporated, the residue codistilled with water and crystallized from water (with addition of 5 parts of ethanol). The product was collected by filtration, washed with ethanol and ether and dried in vacuo; yield 0.55 g (64%) of compound *XIa* (free acid), containing less than 4.5% of the (*R*)-enantiomer (according to HPLC). $[\alpha]_D$ −23.5° (*c* 5.1, 0.1 M HCl). For $C_9H_{14}N_5O_5P$. H_2O (321.2) calculated: 33.65% C, 5.02% H, 21.79% N, 9.65% P; found: 33.56% C, 4.96% H, 21.84% N, 9.79% P.

2-Chloro-9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine (*XIb*)

A mixture of 2-chloroadenine (1.7 g, 10 mmol), cesium carbonate (2.3 g, 7.2 mmol), compound *IX* (6.3 g, 12.4 mmol) and dimethylformamide (50 ml) was stirred at 100 °C for 4 h under exclusion of moisture. The hot reaction mixture was filtered through Celite and the filtrate was taken down. The residue was codistilled with toluene (2 × 25 ml) and extracted with hot chloroform (2 × 100 ml). The extract was filtered, the chloroform evaporated, and the residue chromatographed on a column of silica gel (300 ml) in chloroform and then in chloroform–methanol (95 : 5). The product was dried in vacuo to give compound *Xb* as an amorphous foam (4.3 g, 87%); R_F 0.54 (S3). A solution of this product in 0.1 M methanolic sodium methoxide (100 ml) was allowed to stand at room temperature overnight, the mixture was neutralized with Dowex 50X8 (H^+ form), made alkaline with triethylamine, filtered, the solid material washed with methanol and the filtrate evaporated. The residue was codistilled with ethanol (2 × 25 ml) and dried in vacuo. Acetonitrile (40 ml) and bromotrimethylsilane (4 ml) were added and the mixture was set aside at room temperature overnight. The solvent was again evaporated, the residue codistilled with acetonitrile, mixed with water (100 ml) and made alkaline with ammonia. After standing for 30 min, the solution was taken down and the residue applied onto a column of Dowex 50X8 (H^+ form; 150 ml). After washing out salts with water, the product was eluted with 5% aqueous ammonia, the ammonia eluate was concentrated, the residue dissolved in water (50 ml) and acidified to pH 3.0 with dilute sulfuric acid. After cooling, the deposited product was collected on filter, washed with water, ethanol, ether, and dried in vacuo; yield 1.05 g (33%) of compound *XIb*. For $C_9H_{13}ClN_5O_4P$ (321.7) calculated: 33.60% C, 4.07% H, 11.02% Cl, 21.77% N; found: 33.41% C, 4.05% H, 10.78% Cl, 21.74% N. ^1H NMR spectrum (D_2O + NaOD): 3.51 dd, 1 H (*J*(P,CH) = 9.3, *J*(gem) = 12.7) and 3.68 dd, 1 H (*J*(P,CH) = 9.3) (P–CH_2); 3.55 dd, 1 H (*J*(3″,2′) = 4.9, *J*(gem) = 13.8) and 3.80 dd, 1 H (*J*(3′,2′) = 3.9), (3′-CH_2); 3.83 m, 1 H (2′-CH); 4.24 dd, 1 H (*J*(1″, 2′) = 6.3, *J*(gem) = 14.7) and 4.34 dd, 1 H (*J*(1′,2′) = 4.4) (1′-CH_2); 8.11 s, 1 H (H-8).

(*S*)-2,6-Diamino-9-(3-hydroxy-2-phosphonomethoxypropyl)purine (*XIc*)

A suspension of sodium hydride in paraffin (60%, 0.24 g, 6 mmol) was added to a suspension of 2,6-diaminopurine (0.90 g, 6 mmol) in dimethylformamide (25 ml) and the mixture was stirred at 70 °C for 1 h under exclusion of moisture. A solution of compound *IX* (2.5 g, 5 mmol) in dimethylformamide (5 ml) was added and the mixture was stirred at 100 °C for 24 h under calcium chloride protecting tube. The solvent was evaporated and the residue was allowed to stand with 0.1 M methanolic sodium methoxide (100 ml) at room temperature overnight. The mixture was neutralized with Dowex 50X8 (H^+ form), made alkaline with triethylamine, filtered, the solid material was washed with methanol and the filtrate was concentrated. The residue was codistilled with ethanol (2 × 25 ml) and dried in vacuo. Acetonitrile (30 ml) and bromotrimethylsilane (3 ml) were added and the mixture was allowed to stand at room temperature overnight. The solvent was again evaporated, the residue codistilled with acetonitrile, mixed with water (100 ml) and made alkaline with ammonia. After standing for 30 min, this solution was concentrated and applied onto a column of Dowex 50X8 (H^+ form; 150 ml). The column, containing the precipitated crystalline product,

was washed with water until the salts were removed. Then the product was eluted with 5% aqueous ammonia, the ammonia eluate was evaporated, the residue dissolved in water (50 ml) and acidified with dilute sulfuric acid to pH 3.0. The separated crystalline product was collected by filtration, washed with water, ethanol, ether, and dried; yield 1.03 g (68%) of compound *XIc*, identical (HPLC) with an authentic sample[4].

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

A) *From 4-methoxy-2-pyrimidone* (XII). A mixture of 4-methoxy-2-pyrimidone (*XII*; 0.24 g, 2 mmol), sodium hydride (48 mg, 2 mmol) and dimethylformamide (5 ml) was heated at 80 – 90 °C for 1 h. A solution of compound *IX* (1.16 g, 2 mmol) in dimethylformamide (5 ml) was added and the mixture was further heated at 100 °C for 48 h. The solvent was evaporated and the residue purified by chromatography on silica gel in chloroform with increasing concentration of methanol (final concentration 5%). After evaporation, the obtained product was dissolved in 30% methanolic ammonia (5 ml) and heated in an ampoule at 100 °C for 12 h. The solvent was evaporated, the residue dried in vacuo and allowed to stand with acetonitrile (8 ml) and bromotrimethylsilane (0.7 ml) at room temperature for 24 h. The solvent was again evaporated, the residue codistilled with acetonitrile (5 ml) and mixed with water (20 ml) and triethylamine (2 ml). After standing for 30 min, the solvent was evaporated and the product purified as described for compound *XIa*. Yield of compound *XIVa* was 0.22 g (37%); according to HPLC (vide supra), the product contained less than 3% of the (*R*)-enantiomer. For $C_8H_{14}N_3O_6P \cdot H_2O$ (297.3) calculated: 32.32% C, 5.43% H, 14.14% N, 10.44% P; found: 32.56% C, 4.96% H, 13.84% N, 10.37% P.

B) *From cytosine* (XVa). A mixture of cytosine (237.3 mg, 2.1 mmol), cesium carbonate (340 mg, 2.1 mmol) and dimethylformamide (6 ml) was stirred at 110 °C for 2 h, a solution of compound *IX* (1.16 g, 2.1 mmol) in dimethylformamide (5 ml) was added and the mixture was heated at 100 °C for 72 h. After evaporation in vacuo, the residue was chromatographed on silica gel in chloroform with increasing concentration of methanol. The further work-up (reaction with bromotrimethylsilane, deionization and purification on Dowex) was performed as described in procedure *A*. Yield 0.33 g (52%) of compound *XIVa*, containing (HPLC) less than 1% of the (*R*)-enantiomer.

1-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)-6-azacytosine (*XIVb*)

A stirred mixture of 6-azacytosine (*XVb*; 1.4 g, 12.5 mmol), compound *IX* (6.3 g, 12.4 mmol), cesium carbonate (2.3 g, 7.2 mmol) and dimethylformamide (60 ml) was heated at 100 °C for 16 h (calcium chloride protecting tube). The hot reaction mixture was filtered, the filtrate concentrated, the residue codistilled with toluene (2 × 50 ml) and extracted with hot chloroform (2 × 100 ml). After evaporation of the solvent, the residue was allowed to stand with 0.1 M methanolic sodium methoxide (50 ml) at room temperature overnight. The mixture was neutralized with Dowex 50X8 (H^+ form), made alkaline with triethylamine, filtered and the solid was washed with methanol. The filtrate was concentrated and the residue codistilled with ethanol (2 × 25 ml) and dried in vacuo. Acetonitrile (20 ml) and bromotrimethylsilane (2 ml) were added and the mixture was set aside at room temperature overnight. The solvent was again evaporated, the residue was codistilled with acetonitrile, mixed with water (50 ml) and made alkaline with ammonia. After 30 min, this solution was concentrated and applied onto a column of Dowex 50X8 (H^+ form; 100 ml). Water eluted first the salts and then (with retention) the product. The main UV-absorbing fraction of the product was concentrated in vacuo and the residue was codistilled with ethanol (2 × 25 ml). The product was precipitated from methanol with ether; yield 870 mg (25%) of compound *XIVb* homogeneous according to electrophoresis (E_{Up} = 1.0) and HPLC. For $C_7H_{13}N_4O_6P$ (280.3) calculated: 30.00% C, 4.67% H, 20.00% N, 11.08% P; found: 29.85% C, 5.06% H, 20.29% N, 10.77% P.

(*S*)-2-Amino-9-(3-hydroxy-2-phosphonomethoxypropyl)purine (*XIX*)

A solution of 2-amino-6-chloropurine (*XVI*; 1.0 g, 6 mmol) in dimethylformamide (25 ml) was mixed under exclusion of moisture with 60% suspension of sodium hydride in paraffin (300 mg, 7.5 mmol) and the mixture was stirred at 80 °C for 30 min. A solution of compound *IX* (2.5 g, 5 mmol) in dimethylformamide (5 ml) was added and stirring at 80 °C was continued for 20 h. Acetic acid (0.1 ml) was added and the solvent evaporated. Column chromatography of the residue on silica gel (200 ml) in chloroform afforded compound *XVII* (1.2 g, 43%); R_F 0.85 in S3. This material was hydrogenated in methanol (150 ml) containing hydrochloric acid (0.5 ml) over 10% Pd/C (0.6 g) at room temperature overnight. After filtration through Celite, the filtrate was neutralized with triethylamine and the solvent evaporated. The obtained compound *XVIII* (R_F 0.30, S3) was allowed to stand with 0.1 M methanolic sodium methoxide (50 ml) at room temperature overnight. The mixture was neutralized with Dowex 50X8 (H^+ form), made alkaline with triethylamine, filtered, the solid was washed with methanol, and the filtrate concentrated. The residue was deionized on a column of Dowex 50X8 (100 ml) in 20% methanol. The ammonia eluate was concentrated, the residue codistilled with ethanol (2 × 25 ml) and dried in vacuo. Acetonitrile (30 ml) and bromotrimethylsilane (3 ml) were added and the mixture was set aside at room temperature overnight. The solvent was again evaporated, the residue codistilled with acetonitrile, mixed with water (100 ml) and made alkaline with ammonia. After 30 min, the solvent was evaporated and the residue deionized on a column of Dowex 50X8 (H^+ form; 100 ml). The residue after evaporation of the ammonia eluate was dissolved in water (25 ml), adjusted to pH 9 with ammonia and applied onto a column of Dowex 1X2 (acetate form; 100 ml). The column was washed with 0.05 M acetic acid to drop of UV absorption and then with a gradient (0.05 – 0.3 mol l^{-1}) of acetic acid (1 l each). The product-containing fraction was concentrated in vacuo, the residue was codistilled with water (3 × 20 ml) and crystallized from aqueous ethanol to give 500 mg (31% calculated on the starting *IX*) of compound *XIX*, identical with an authentic sample[4]. For $C_9H_{14}N_5O_4P \cdot H_2O$ (305.3) calculated: 35.40% C, 5.28% H, 22.95% N, 10.16% P; found: 35.33% C, 5.28% H, 23.09% N, 9.16% P. ^{1}H NMR spectrum (D_2O + NaOD): 3.53 dd, 1 H (*J*(P,CH) = 9.8, *J*(gem) = 12.4) and 3.57 dd, 1 H (*J*(P,CH) = 8.8) (P–CH_2); 3.51 dd, 1 H (*J*(3″,2′) = 5.1, *J*(gem) = 12.4) and 3.84 dd, 1 H (*J*(3′,2′) = 3.7); 3.84 m, 1 H (2′-CH); 4.28 dd, 1 H (*J*(1″,2′) = 6.4, *J*(gem) = 14.6) and 4.34 dd, 1 H (*J*(1′,2′) = 4.6) (1′-CH_2); 8.24 s, 1 H (H-8); 8.54 s, 1 H, (H-6).

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