ACYCLIC NUCLEOSIDE AND NUCLEOTIDE ANALOGS DERIVED FROM 2-AZAADENINE*

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Alkylation of cesium salt of 2-azaadenine with appropriate synthons afforded the following acyclic analogs of nucleosides and nucleotides: (*S*)-9-(2,3-dihydroxypropyl)-2-azaadenine (*VI*), 3-(2-aza-adenin-9-yl)-2-hydroxypropanoic acid (*VIII*), 9-(2-phosphonomethoxyethyl)-2-azaadenine (*Xb*), (*R*)-9-(2-phosphonomethoxypropyl)-2-azaadenine (*XIb*) and (*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)-2-azaadenine (*XVIb*). In some cases the N-2 isomers were also isolated.

Studies of acyclic nucleoside and nucleotide analogs have shown high specificity of their biological effect with respect to the purine base. It appeared that a limited retention of the biological effect can be achieved by replacement of a methine grouping with an atom of nitrogen and vice versa in the so-called aza or deaza derivatives. Recently we described syntheses and some biological effects of 1-deaza- and 3-deazapurines[2] and 8-azapurines[3].

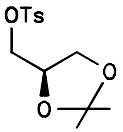
Although 2-azaadenine (*I*) and 2-azahypoxanthine are known to inhibit the growth of microbial as well as mammalian cells[4–7], the biological activity of their derivatives has been studied relatively little[8,9] and no systematic studies in this respect are available. Our present study is aimed at the preparation of 2-aza analogs of such acyclic types of purine nucleoside and nucleotide analogs, whose "parent" adenine derivatives possess interesting biological properties[10–12].

As concerns acyclic nucleoside analogs, we focused our attention to analogs of (*S*)-9-(2,3-dihydroxy- propyl)adenine (DHPA), *erythro*-(2*R*,3*R*)-4-(adenin-9-yl)-2,3-dihydroxy-butanoic acid (eritadenin) and 3-(adenin-9-yl)-2-hydroxypropanoic acid (AHPA), i.e. compounds which, as inhibitors of enzyme SAH-hydrolase, have a broad spectrum of biological activity[10]. Our previous studies[2] have proven high specificity of this enzyme

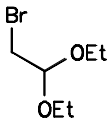
* A part of this work has been published in an abbreviated form[1].

towards the adenine base and only an exceptional possibility of its replacement by the 3-deazaadenine moiety without loss of activity.

Because of variability of the side-chain, as a general approach we have chosen the alkylation of the cesium salt of 2-azaadenine with a suitable synthon (*II* – *IV*) and, if needed, a modification of the side-chain. Surprisingly, in one case we isolated only the N-2 isomer.



II



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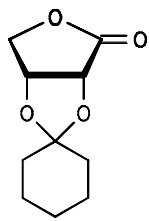

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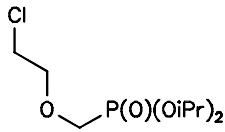
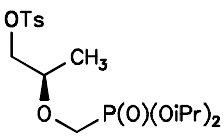


XII



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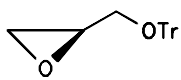
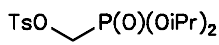


XIV



XVII

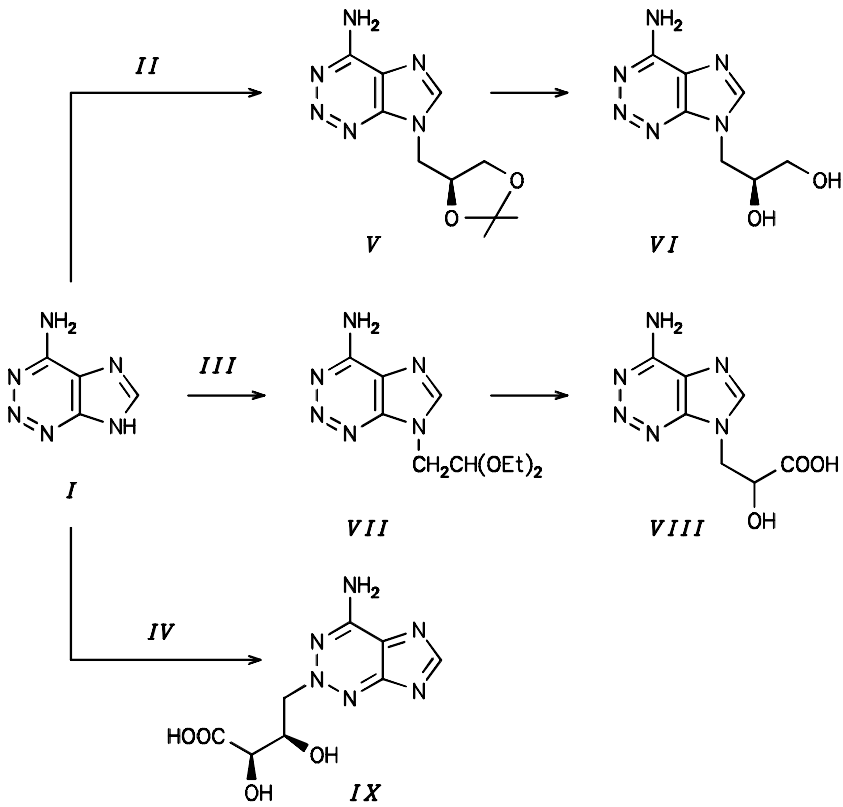
2-Azaadenine (*I*) was synthesized using the described[4,13] procedure starting from adenine which was oxidized with *m*-chloroperoxybenzoic acid to the N-1 oxide. The pyrimidine ring was subjected to acid cleavage and after reduction it was again cyclized by diazotization reaction with simultaneous introduction of nitrogen atom into position 2 to give the 1,2,3-triazine ring.

The (*S*)-9-(2,3-dihydroxypropyl) derivative *VI* was prepared starting from cesium salt of 2-azaadenine formed in situ from the base and cesium carbonate in dimethylformamide[2]. Reaction of the salt with (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*II*) afforded isopropylidene derivative *V* which upon acid hydrolysis gave the desired compound *VI* (Scheme 1). 3-(2-Azaadenin-9-yl)-2-hydroxypropanoic acid (*VIII*) was synthesized by alkylation of cesium salt of 2-azaadenine with bromoacetaldehyde diethyl acetal (*III*) followed by modification of the side-chain in the obtained intermediate *VII* by cyanohydrin synthesis[14]. In both cases we obtained the N-9 isomers with only negligible amounts of other regioisomers.

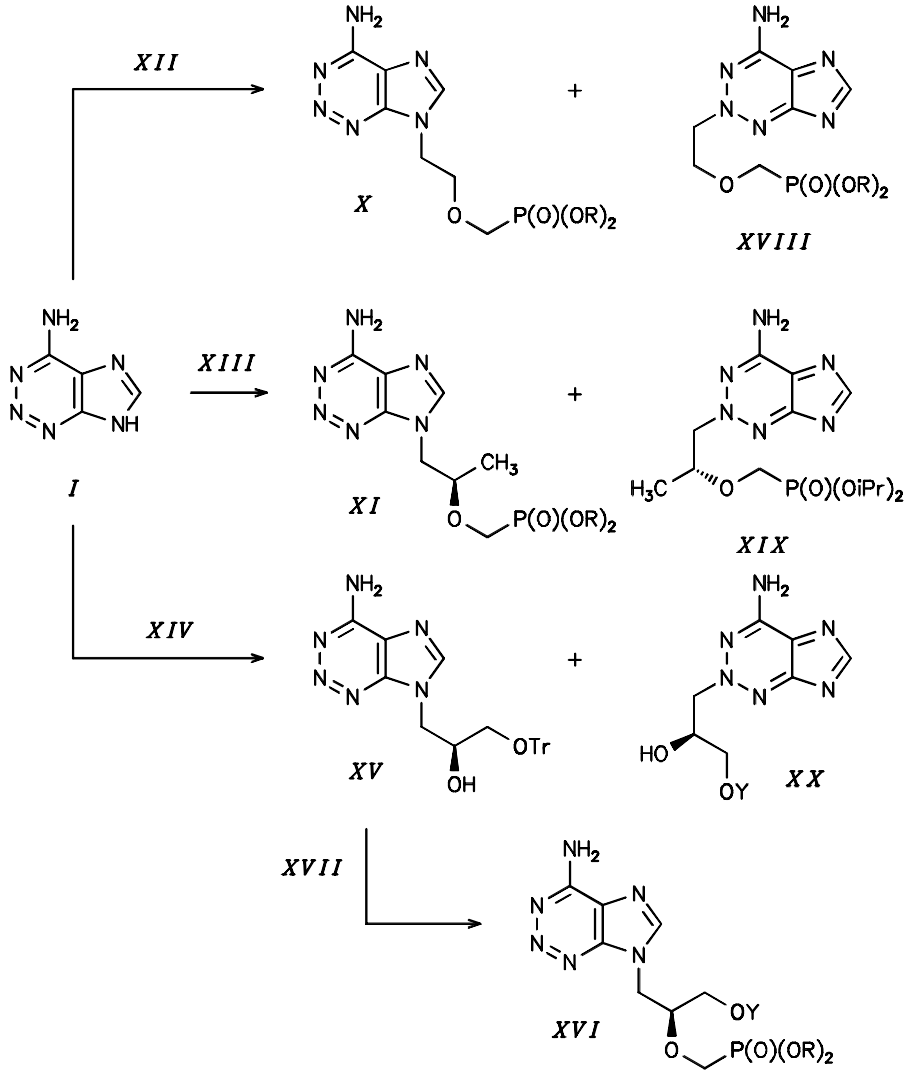
On the contrary, the attempted synthesis of *erythro*-(2*R*,3*R*)-4-(2-azaadenin-9-yl)-2,3-dihydroxybutanoic acid by reaction of cesium salt of 2-azaadenine with 2,3-*O*-cyclohexylidene-D-erythronolactone[15] (*IV*) did not give any N-9 isomer; the only product isolated from the reaction mixture was the N-2 isomer *IX* (vide infra).

Further group of 2-azapurine compounds that we prepared in this connection (Scheme 2) were derivatives of the three phosphonate acyclic nucleotide analogs whose adenine derivatives[11,12] (9-(2-phosphonomethoxyethyl)adenine (PMEA), (*R*)-9-(2-phosphonomethoxypropyl)adenine (PMPA) and (*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine (HPMPA)) exhibit significant antiviral effects. Also in these cases high base-specificity of the biological effect, and limited possibility of replacing adenine with the 8-azaadenine system without loss of activity, have been already proven[3].

9-(2-Phosphonomethoxyethyl)-2-azaadenine (*Xb*) and (*R*)-9-(2-phosphonomethoxypropyl)-2-azaadenine (*XIb*) were prepared by alkylation of cesium salt of 2-azaadenine with bis(2-propyl) 2-chloroethoxymethylphosphonate (*XII*) and synthon *XIII*, respectively. In both cases, the ester protective groups were removed by action of bromotrimethylsilane.



SCHEME 1



In formulae *X*, *XI*, *XVI*, *XVIII*, *XX* :

a, R = iPr; Y = Tr

b, R = H; Y = H

SCHEME 2

In the synthesis of (*S*)-9-(2-phosphonomethoxy-3-hydroxypropyl)-2-azaadenine (*XVIb*) the phosphonate functionality was introduced only into a suitably protected 2,3-dihydroxypropyl derivative: 2-azaadenine was converted into compound *XV* by reaction with (*S*)-1-*O*-tritylglycidol (*XIV*) in the presence of catalytic amount of cesium carbonate. The amino group in derivative *XV* was protected by the amidine group and the obtained product was alkylated with bis(2-propyl) tosyloxymethylphosphonate (*XVII*). After deprotection of the amino group by alkaline hydrolysis, the obtained compound *XVIa* was treated with bromotrimethylsilane[16] to remove simultaneously both the trityl and the ester groups.

As expected, also in these cases the alkylation gave predominantly the N-9 isomers. In addition, we identified small amounts of the N-2 isomers *XVIII – XX*. After desalting, the products *Xb*, *XIb*, *XVIb* and *XVIIIb* were isolated as the free acids.

Discussion of NMR Spectra

The structure of the alkylation products was studied by ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectra (see Experimental) characterize structure of the individual groups but afford only very limited information about the structure of the base (protons H-8 and NH_2). Comparison of the data for the N-9 and N-2 isomers shows that the position of the substituent has practically no influence on the chemical shift of the H-8 proton, whereas signals of alkyl protons in positions 1′ and 2′ of the N-2 isomers are shifted downfield (0.4 ppm and 0.3 ppm, respectively). On the other hand, the ^{13}C chemical shifts differ markedly for both groups of isomers (see Table I). Spectra of compounds, assigned the N-2 isomer structure, exhibit significant downfield shifts of the alkyl carbon atom C-1′ (about 22 ppm) as well as of all the carbon atoms in the base (C-8 about 16 ppm, C-4 about 11 ppm, C-5 about 7 ppm), except for the C-6 atom (about 0 ppm). A similar, though somewhat smaller, downfield effect on the C-1′ alkyl carbon atom (about 10 ppm) was observed with 8-alkyl derivatives of 8-azaadenine and 8-azaguanine compared with the N-9 or N-7 isomers[17] and was also described for the anomeric carbon atom C-1′ in 2′,3′-dideoxyribonucleosides of 8-azaguanine[18,19]. The greater downfield effect on C-1′ described in the present study is apparently connected with different geometry of the =N–N–N= fragment in six-membered and five-membered rings and the resulting differences in the deshielding effects.

An unequivocal decision between the N-9 and N-2 alkyl regioisomers has been done on the basis of proton-coupled ^{13}C NMR spectra and of selective proton decoupling experiments for the selected pair of compounds *VI* and *XXb*. Selective decoupling of protons H-8, NH_2, H-1a′ and H-1b′ determined the individual vicinal coupling constants ^3J(C,H) as shown in Fig. 1. Whereas for both the isomers we observed interactions of the NH_2 protons with the C-5 carbon atom, of the H-8 proton with the C-4 and C-5 atoms, and a singlet of C-6, a significantly different fine-splitting of the C-4 and C-8 signals was observed. The signals of both these carbon atoms in the N-9 isomer are

TABLE I
Selected carbon-13 chemical shifts of 2-azaadenine nucleoside and nucleotide analogs

Compound	Solvent	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′
				N-9 isomers				
VI	DMSO	147.57	116.54	152.94	145.76	47.83	69.95	63.92
VIII	DMSO	147.08	116.10	152.67	145.24	47.02	68.51	173.04
Xa	DMSO	147.04	116.20	152.77	144.82	43.56	70.19	–
Xb	D_2O	147.68	118.08	153.64	147.65	45.40	71.12	–
XIa	DMSO	147.28	115.96	152.71	145.07	47.74	75.25	16.79
XIb	D_2O	148.06	117.94	153.73	148.00	49.53	76.74	17.17
XVIb	DMSO	147.14	116.03	152.68	144.84	44.63	78.43	62.68
XV	DMSO	147.19	116.20	152.68	145.12	47.63	67.84	65.82
				N-2 isomers				
IX	DMSO	158.08	123.20	153.05	161.30	69.37	72.04	71.97
XVIIIb	D_2O	157.84	123.79	155.66	161.13	67.27	71.06	–
XXa	DMSO	158.12	123.31	152.99	161.56	70.10	69.12	65.83
XXb	DMSO	158.16	123.18	153.04	161.40	70.32	71.18	63.65

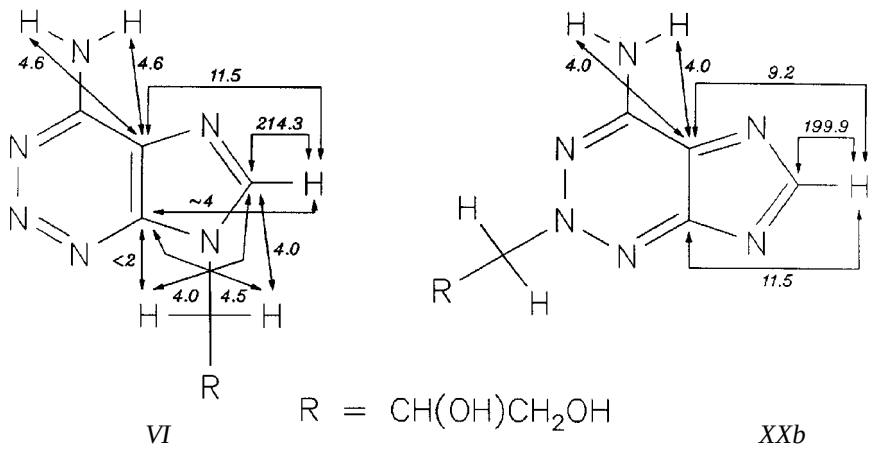


FIG. 1
The observed carbon–proton coupling constants (indicated with arrows) of 2-azaadenine carbon atoms in compound *VI* and *XXb*

split due to vicinal coupling with the Ha-1′ and Hb-1′ protons whereas in the N-2 isomer these interactions are absent. Their absence is just the proof of the N-2 isomer because in it the alkyl protons Ha-1′ and Hb-1′ are separated by four bonds from the nearest carbon atoms in the base (C-4 and C-6). In the case of the N-1 and N-3 isomer we should observe their vicinal interactions with the C-6 and the C-4 carbon atom, respectively.

Biological Activity

The choice of biological assay systems for evaluation of the synthesized compounds followed our previous knowledge on the biological activities of adenine derivatives. The in vitro inhibition of SAH-hydrolase and the cytostatic assays were performed by Dr I. Votruba of this Institute, the antiviral activities were determined at the Rega Institute (Professor E. De Clercq), Catholic University Leuven, Belgium.

The inhibition of C–S bond scission in *S*-adenosyl-L-homocysteine, catalyzed by SAHase from L-1210 cells[10], was evaluated as v_i/v_0 values which reflect the ratio of initial reaction rates in the presence and absence of the compound under study. Of the series examined so far, only compound *VIII* showed inhibitory activity at concentration $1.5 \cdot 10^{-6}$ mol l^{-1} ($v_i/v_0 = 0.25$). Nonetheless, even in this case, replacement of the methine group at position 2 by nitrogen resulted in a lower inhibitory activity.

Antiviral activity against retroviruses HIV-1, HIV-2 and MSV was assayed in tissue cultures of human MT-4 cells and CEM cells; none of the compounds tested had any appreciable antiviral activity. Compound *XVIb* exhibited significant antiviral effect against herpesviruses (HSV-1, HSV-2) at concentration $6.6 \cdot 10^{-5} - 6.6 \cdot 10^{-6}$ mol l^{-1}. The details will be published in a separate communication.

Cytostatic activity was assayed in vitro in L1210 mouse leukemia cells. None of the above compounds displayed any appreciable cytostatic activity at concentration $1 \cdot 10^{-5}$ mol l^{-1}.

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography was performed on Silufol UV 254 sheets, preparative thin-layer chromatography was carried out on 40 × 17 × 0.4 cm plates of silica gel with UV-indicator (Kavalier, Votice, The Czech Republic). Column chromatography was performed on silica gel (30 μm) of the same provenience.

Solutions were deionized on Dowex 50 X 8 (100 – 200 mesh, H^+ form) in the following manner: after application of the mixture, the column was washed with water until UV absorption (254 nm) of the eluate dropped to the original value and the product was then eluted with 2.5% aqueous ammonia. Chromatography on Dowex 1 X 2 (100 – 200 mesh, acetate form) was carried out so that the column was first washed with water to drop of the UV absorption (254 nm) to the original value and

the compound was then eluted with a linear gradient water–acetic acid or with 0.5 – 1 mol l^{-1} acetic acid.

Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using the EI (electron energy 70 eV) and FAB (ionization with Xe, accelerating voltage 8 kV) techniques. Proton NMR spectra were taken on Varian UNITY-200 (200 MHz) and Varian UNITY-500 (500 MHz) instruments in hexadeuteriodimethyl sulfoxide or in deuterium oxide with tetramethylsilane or sodium disilapentanesulfonate (DSS) as the respective internal standards. ^{13}C NMR spectra were measured on a Varian UNITY-500 (125.7 MHz) instrument, the chemical shifts were referenced to the solvent signal ($\delta(CD_3SOCD_3)$ = 39.7 ppm) or, for solutions in deuterium oxide, to dioxane as the external standard (δ(dioxane) = 66.86 ppm).

Compounds and reagents: dimethylformamide and acetonitrile were dried by distillation from phosphorus pentoxide and stored over molecular sieves. Bromotrimethylsilane, bromoacetaldehyde diethyl acetal and cesium carbonate were Fluka products.

2-Azaadenine (*I*)

Azaadenine was prepared according to the literature[4,13] as an amorphous solid without melting point. For $C_4H_4N_6$ (135.1) calculated: 35.56% C, 2.98% H, 62.20% N; found: 35.81% C, 3.01% H, 61.56% N. ^1H NMR spectrum: 11.50 br, 1 H (NH); 8.48 s, 1 H (H-8); 7.65 brs, 2 H (NH_2).

(*S*)-9-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-2-azaadenine (*V*)

A mixture of 2-azaadenine (*I*) (1.0 g, 7.4 mmol), cesium carbonate (1.3 g, 4 mmol), (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane[10](*II*) (2.3 g, 8 mmol) and dimethylformamide (30 ml) was heated at 120 °C for 6 h (calcium chloride protecting tube). After evaporation of the solvent and codistillation with toluene and methanol, the residue was dissolved in methanol, adsorbed on silica gel and chromatographed on a column of silica gel (100 ml). The product was eluted with a chloroform–methanol mixture (95 : 5). Crystallization from ethanol afforded 0.6 g (30%) of compound *V*, m.p. 231 – 233 °C. For $C_{10}H_{14}N_6O_2$ (250.3) calculated: 47.99% C, 5.64% H, 33.58% N; found: 47.76% C, 5.63% H, 33.26% N. ^1H NMR spectrum: 8.42 s, 1 H (H-8); 7.75 brs, 2 H (NH_2); 4.55 tt, 1 H, ΣJ = 22.2 (H-2′); 4.50 dd, 1 H, J(1a′,2′) = 4.2, J(gem) = 14.2 (Ha-1′); 4.41 dd, 1 H, J(1b′,2′) = 6.6, J(gem) = 14.2 (Hb-1′); 4.07 dd, 1 H, J(3a′,2′) = 6.3, J(gem) = 8.8 (Ha-3′); 3.81 dd, 1 H, J(3b′,2′) = 5.1, J(gem) = 8.8 (Hb-3′); 1.25 and 1.22 2 × s, 2 × 3 H (CH_3). Mass spectrum (FAB), *m/z* (rel.%): 250 (80) $[M]^+$.

(*S*)-9-(2,3-Dihydroxypropyl)-2-azaadenine (*VI*)

A solution of (*S*)-9-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-2-azaadenine (*V*) (0.54 g, 2.16 mmol) in 0.25 M H_2SO_4 (30 ml) was set aside at room temperature for 15 h. The mixture was neutralized with saturated solution of barium hydroxide, briefly boiled and filtered through Celite. After evaporation of the solvent, the product was crystallized from ethanol; yield 0.4 g (88%) of compound, m.p. 215 – 218 °C. For $C_7H_{10}N_6O_2$ (210.2) calculated: 39.99% C, 4.79% H, 39.98% N; found: 39.64% C, 4.67% H, 39.62% N. ^1H NMR spectrum: 8.37 s, 1 H (H-8); 7.70 brs, 2 H (NH_2); 5.15 d, 1 H, J(OH,CH) = 5.5 (OH); 4.88 t, 1 H, $J(OH,CH_2)$ = 5.6 (OH); 4.48 dd, 1 H, J(1a′,2′) = 3.5, J(gem) = 14.0 (Ha-1′); 4.17 dd, 1 H, J(1b′,2′) = 8.5, J(gem) = 14.0 (Hb-1′); 3.91 m, 1 H, ΣJ = 28.8 (H-2′); 3.44 pent, 1 H, ΣJ = 22.0 (Ha-3′); 3.37 pent, 1 H, ΣJ = 23.0 (Hb-3′). Mass spectrum (FAB), *m/z* (rel.%): 211 (40) $[M + H]^+$.

9-(2,2-Diethoxyethyl)-2-azaadenine (*VII*)

A stirred mixture of 2-azaadenine (*I*; 1.2 g, 8.9 mmol), cesium carbonate (1.5 g, 4.6 mmol), bromoacetaldehyde diethyl acetal (1.4 ml, 9 mmol) and dimethylformamide (20 ml) was heated at 115 °C for 16 h (calcium chloride protecting tube). After evaporation of the solvent and codistillation with toluene and methanol, the residue was dissolved in methanol, adsorbed on silica gel and chromatographed on a column of silica gel. Elution with chloroform, containing 2% of methanol, followed by crystallization from ethanol, afforded 0.4 g (20%) of compound *VII*, m.p. 212 – 217 °C. For $C_{10}H_{16}N_6O_2$ (252.3) calculated: 47.61% C, 6.39% H, 33.31% N; found: 47.42% C, 6.29% H, 32.95% N. ^{1}H NMR spectrum: 8.39 s, 1 H (H-8); 7.74 brs, 2 H (NH_2); 4.42 d, 2 H, J(1′,2′) = 5.1 (H-1′); 4.92 t, 1 H, J(2′,1′) = 5.1 (H-2′); 3.35 and 3.65 2 × dq, 2 × 2 H, J(CH_2,CH_3) = 7.1, J(gem) = 9.5 (OCH_2); 1.01 t, 6 H, J(CH_3,CH_2) = 7.1 (CH_3). Mass spectrum (FAB), *m*/*z* (rel.%): 253 (90) $[M + H]^+$.

3-(2-Azaadenin-9-yl)-2-hydroxypropanoic Acid (*VIII*)

A mixture of compound *VII* (0.3 g, 1.2 mmol), water (10 ml) and concentrated hydrochloric acid (0.3 ml) was heated at 80 °C for 5 h. The reaction mixture was cooled to −5 °C and sodium cyanide (0.34 g) and acetic acid (ca 0.2 ml) were successively added to neutrality under stirring and cooling. The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. After addition of concentrated hydrochloric acid (5 ml), the mixture was refluxed for 3 h, the solvent was evaporated, and the residue was codistilled with water and desalted on Dowex 50 X 8 (100 ml). The ammonium salt of the product was chromatographed on Dowex 1 X 2 (50 ml). After washing the column with water, the product was eluted with a linear gradient of acetic acid (0 – 0.5 mol l^{-1}, à 1 liter). The product-containing fractions were evaporated, the residue was codistilled with water and crystallized from ethanol to give 0.1 g (37%) of compound *VIII*, m.p. 247 – 251 °C. For $C_7H_8N_6O_3$ (224.2) calculated: 37.50% C, 3.59% H, 37.49% N; found: 37.28% C, 3.70% H, 37.12% N. ^{1}H NMR spectrum: 8.37 s, 1 H (H-8); 7.73 brs, 2 H (NH_2); 5.87 brs, 1 H (COOH); 4.62 m, 1 H (H-2′); 4.47 m, 2 H (H-1′). Mass spectrum (FAB), *m*/*z* (rel.%): 225 (20) $[M + H]^+$.

(2*R*,3*R*)-4-(2-Azaadenin-2-yl)-2,3-dihydroxybutanoic Acid (*IX*)

A mixture of 2-azaadenine (*I*; 1.4 g, 10 mmol), sodium hydride (60% dispersion, 1.75 g, 5.3 mmol) and dimethylformamide (40 ml) was stirred at 80 °C for 30 min. 2,3-*O*-Cyclohexylidene-D-erythronolactone[18] (*IV*; 3.3 g, 16.8 mmol) was added and the reaction mixture was stirred at 125 °C for 2 days. After evaporation of the solvent and codistillation with toluene, the residue was refluxed with 85% formic acid (50 ml) for 6 h. The mixture was taken down, the residue was codistilled with water and desalted on Dowex 50 X 8 (150 ml). The ammonium salt of the product was then chromatographed on Dowex 1 X 2 (50 ml). After washing with water, the product was eluted with 1 M acetic acid. The product-containing fractions were evaporated, the residue was codistilled with water and chromatographed on a column of Sephadex A 25 (HCO_3^- form), elution with linear gradient 0.02 – 0.2 M TEAB, à 1 liter, 100 ml). The product-containing fraction was again converted into the free acid on a column of Dowex 1 X 2 (50 ml). Crystallization from aqueous ethanol afforded 0.2 g (8%) of white crystalline product *IX*, m.p. 232 – 234 °C. For $C_8H_{10}N_6O_4$ (254.2) calculated: 37.83% C, 3.97% H, 33.09% N; found: 37.62% C, 3.84% H, 32.73% N. ^{1}H NMR spectrum: 8.41 s, 1 H (H-8); 7.89 brs, 2 H (NH_2); 6.30 brs, 1 H (COOH); 4.84 dd, 1 H, J(1a′,2′) = 2.4, J(gem) = 12.2 (Ha-1′); 4.60 dd, 1 H, J(1b′,2′) = 9.8, J(gem) = 12.2 (Hb-1′); 4.45 ddd, 1 H, J(2′,1a′) = 2.4, J(2′,1b′) = 9.8, J(2′,3′) = 5.2 (H-2′); 4.09 d, 1 H, J(3′,2′) = 5.2 (H-3′). Mass spectrum (FAB), *m*/*z* (rel.%): 255 (65) $[M + H]^+$.

9-(2-Phosphonomethoxyethyl)-2-azaadenine (*Xb*)

A stirred mixture of 2-azaadenine (*I*; 2.7 g, 20 mmol), cesium carbonate (3.3 g, 10 mmol) and dimethylformamide (50 ml) was heated at 100 °C for 1 h (calcium chloride tube). Bis(2-propyl) 2-chlorethoxymethylphosphonate[11] (*XII*; 5.4 g, 21 mmol) was added and the reaction mixture was heated at 120 °C for 16 h. After evaporation of the solvent and codistillation with toluene and methanol, the residue was dissolved in methanol, adsorbed on silica gel and chromatographed on a column of silica gel. Elution with 2% methanol in chloroform, followed by crystallization from ethanol, afforded 1.2 g (18%) of compound *Xa* containing small amount of the N-2 isomer *XVIIIa*. ^{1}H NMR spectrum (*Xa*): 8.42 s, 1 H (H-8); 7.72 brs, 2 H (NH_2); 4.52 t, 2 H, J(1′,2′) = 5.1 (H-1′); 4.45 dsept, 2 H, J(POCH) = 7.6, J(CH,CH_3) = 6.1 (POCH); 3.97 t, 2 H, J(2′,1′) = 5.1 (H-2′); 3.79 d, 2 H, J(PCH) = 8.3 (PCH_2); 1.14 and 1.09, 2 × d, 2 × 6 H, J(CH_3,CH) = 6.1 (CH_3).

The crude product *Xa* (1.2 g, 3.35 mmol) was mixed with acetonitrile (30 ml) and bromotrimethylsilane (3 ml) and the mixture was stirred in a stoppered flask at room temperature for 24 h. After evaporation of the solvent, the residue was codistilled with acetonitrile, dissolved in water (50 ml) and adjusted to pH 12 with triethylamine. The solvent was evaporated, the residue was desalted on Dowex 50 X 8 (100 ml) and chromatographed on Dowex 1 X 2 (50 ml). After washing with water, the side-product *XVIIIb* (N-2 isomer) was eluted with 1 M acetic acid; the N-9 isomer *Xb* was then obtained from the Dowex by extraction with boiling 2 M acetic acid. Both fractions were separately taken down and the residues were codistilled with water and crystallized from aqueous ethanol. Yield 0.4 g (44%) of compound *Xb*, m.p. 229 – 231 °C. For $C_7H_{11}N_6O_4P$ (274.2) calculated: 30.68% C, 4.05% H, 30.67% N, 11.30% P; found: 30.86% C, 4.16% H, 30.40% N, 11.68% P. ^{1}H NMR spectrum (D_2O): 8.55 s, 1 H (H-8); 4.62 t, 2 H, J(1′,2′) = 5.0 (H-1′); 4.03 t, 2 H, J(2′,1′) = 5.0 (H-2′); 3.60 d, 2 H, J(PCH) = 8.8 (PCH_2). Mass spectrum (FAB), *m*/*z* (rel.%): 275 (80) $[M + H]^+$. Further we obtained 0.1 g (11%) of the N-2 isomer *XVIIIb*. ^{1}H NMR spectrum (D_2O): 8.45 s, 1 H (H-8); 4.84 t, 2 H, J(1′,2′) = 5.0 (H-1′); 4.26 m, 2 H (H-2′); 3.52 d, 2 H, J(PCH) = 8.3 (PCH_2). Mass spectrum (FAB), *m*/*z* (rel.%): 275 (100) $[M + H]^+$.

Bis(2-propyl) (*R*)-9-(2-Phosphonomethoxypropyl)-2-azaadenine (*XIa*)

A stirred mixture of 2-azaadenine (*I*; 1.0 g, 7.4 mmol), cesium carbonate (1.2 g, 3.7 mmol) and dimethylformamide (45 ml) was heated at 120 °C for 1 h (calcium chloride protecting tube). After addition of bis(2-propyl) (*R*)-2-1-(tosyloxy)propoxymethylphosphonate[11] (*XIII*; 2.0 g, 4.9 mmol), the heating at 120 °C was continued for 24 h. The reaction mixture, containing in addition to the desired N-9 isomer *XIa* also negligible amount of the N-2 isomer *XIX*, was taken down, the residue was codistilled with toluene and methanol and the compound *XIa* was obtained by chromatography on a column of silica gel (2% methanol in chloroform). Yield 0.6 g (33%) of amorphous compound *XIa*. ^{1}H NMR spectrum: 8.37 s, 1 H (H-8); 7.22 brs, 2 H (NH_2); 4.40 – 4.50 m, 3 H (Ha-1′ and POCH); 4.34 dd, 1 H, J(1b′,2′) = 7.1, J(gem) = 14.4 (Hb-1′); 4.04 m, ΣJ = 29.0 (H-2′); 3.82 dd, 1 H, J(PCH) = 9.0, J(gem) = 13.7 (PCH_2); 3.71 dd, 1 H, J(PCH) = 9.5, J(gem) = 13.7 (PCH_2); 1.18 d, 3 H, J = 6.3 (CH_3); 1.133 d, 6 H, J = 6.1 (CH_3); 1.129 d, 3 H, J = 6.3 (CH_3); 1.08 d, 3 H, J = 6.3 (CH_3). Mass spectrum (FAB), *m*/*z* (rel.%): 373 (100) $[M + H]^+$.

(*R*)-9-(2-Phosphonomethoxypropyl)-2-azaadenine (*XIb*)

A mixture of compound *XIa* (0.5 g, 1.3 mmol), acetonitrile (15 ml) and bromotrimethylsilane (1.2 ml) was stirred in a stoppered flask at room temperature for 24 h. The solvent was evaporated, the residue codistilled with acetonitrile and dissolved in water (20 ml). The solution was adjusted to pH 12, the solvent was evaporated and the residue desalted on Dowex 50 X 8 (50 ml). The obtained am-

monium salt of the product was then chromatographed on Dowex 1 X 2 (50 ml). After washing with water and 1 M acetic acid, the product was obtained from the Dowex by extraction with 2 M acetic acid. Evaporation, codistillation with water and crystallization from aqueous ethanol afforded 0.15 g (40%) of white crystalline product *XIb*, m.p. 214 – 216 °C. For $C_8H_{13}N_6O_4P$ (288.2) calculated: 33.36% C, 4.55% H, 29.18% N, 10.75% P; found: 33.48% C, 4.62% H, 28.84% N, 10.96% P. ^{1}H NMR spectrum (D_2O): 8.55 s, 1 H (H-8); 4.60 dd, 1 H, *J*(1a′,2′) = 4.0, *J*(gem) = 14.9 (Ha-1′); 4.43 dd, 1 H, *J*(1b′,2′) = 6.6, *J*(gem) = 14.9 (Hb-1′); 4.05 m, 1 H (H-2′); 3.67 brt, 1 H (PCH_2); 3.50 dd, 1 H, *J*(PCH) = 10.0, *J*(gem) = 12.0 (PCH_2); 1.20 d, 3 H, *J* = 5.6 (CH_3). Mass spectrum (FAB), *m*/*z* (rel.%): 289 (100) $[M + H]^+$.

(*S*)-9-(3-*O*-Trityl-2,3-dihydroxypropyl)-2-azaadenine (*XV*)

A stirred mixture of 2-azaadenine (*I*; 2.7 g, 20 mmol), cesium carbonate (0.2 g, 0.6 mmol) and dimethylformamide (100 ml) was heated at 120 °C for 1 h (calcium chloride protective tube). 1-*O*-Trityl-(*R*)-glycidol[20] (*XIV*; 7.6 g, 24 mmol) was added and the mixture was heated at 125 °C for 4 h. The reaction mixture was taken down, the residue was codistilled with toluene and methanol, dissolved in methanol, adsorbed on silica gel and chromatographed on a column of silica gel. Elution with 3% methanol in chloroform, followed by crystallization from ethanol, gave 2.65 g (30%) of the N-9 isomer *XV*, m.p. 145 – 146 °C. For $C_{26}H_{24}N_6O_2$ (452.5) calculated: 69.09% C, 5.35% H, 18.59% N; found: 69.32% C, 5.41% H, 18.28% N. ^{1}H NMR spectrum (*XV*): 8.32 s, 1 H (H-8); 7.69 brs, 2 H (NH_2); 7.39 d, 6 H (arom.); 7.32 t, 6 H (arom.); 7.26 t, 3 H (arom.); 5.43 d, 1 H, *J*(OH,CH) = 5.6 (OH); 4.51 dd, 1 H, *J*(1a′,2′) = 3.9, *J*(gem) = 13.7 (Ha-1′); 4.30 dd, 1 H, *J*(1b′,2′) = 8.3, *J*(gem) = 13.7 (Hb-1′); 4.19 m, 1 H, Σ*J* = 28.8 (H-2′); 3.05 dd, 1 H, *J*(3a′,2′) = 5.1, *J*(gem) = 9.5 (Ha-3′); 2.91 dd, 1 H, *J*(3b′,2′) = 5.9, *J*(gem) = 9.5 (Hb-3′). Mass spectrum (FAB), *m*/*z* (rel.%): 453 (20) $[M + H]^+$.

The N-2 isomer *XXa* was isolated as side-product (0.4 g, 4.5%). ^{1}H NMR spectrum: 8.41 s, 1 H (H-8); 7.87 brs, 2 H (NH_2); 7.39 d, 6 H (arom.); 7.33 t, 6 H (arom.); 7.26 t, 3 H (arom.); 5.46 d, 1 H, *J*(OH,CH) = 5.9 (OH); 4.88 dd, 1 H, *J*(1a′,2′) = 3.9, *J*(gem) = 12.4 (Ha-1′); 4.56 dd, 1 H, *J*(1b′,2′) = 9.0, *J*(gem) = 12.4 (Hb-1′); 4.42 m, 1 H, Σ*J* = 29.5 (H-2′); 3.12 dd, 1 H, *J*(3a′,2′) = 4.6, *J*(gem) = 9.5 (Ha-3′); 3.06 dd, 1 H, *J*(3b′,2′) = 6.1, *J*(gem) = 9.5 (Hb-3′). Mass spectrum (FAB), *m*/*z* (rel.%): 453 (25) $[M + H]^+$.

(*S*)-2-(2,3-Dihydroxypropyl)-2-azaadenine (*XXb*)

A mixture of compound *XXa* (0.3 g, 0.7 mmol), acetonitrile (10 ml) and bromotrimethylsilane (0.5 ml) was stirred in a stoppered flask at room temperature for 24 h. The solvent was evaporated and the residue was dissolved in a mixture of 5% aqueous triethylamine (2 ml) and 80% aqueous ethanol (10 ml). After evaporation, the residue was dissolved in water (30 ml) and washed three times with ether. Crystallization from ethanol afforded 0.12 g (87%) of compound *XXb*. For $C_7H_{10}N_6O_2$ (210.2) calculated: 39.99% C, 4.79% H, 39.98% N; found: 39.75% C, 4.72% H, 39.59% N. ^{1}H NMR spectrum: 8.40 s, 1 H (H-8); 7.97 brs, 2 H (NH_2); 5.19 d, 1 H, *J*(OH,CH) = 5.0 (OH); 4.93 t, 1 H, *J*(OH,CH_2) = 5.6 (OH); 4.86 dd, 1 H, *J*(1a′,2′) = 3.2, *J*(gem) = 12.0 (Ha-1′); 4.45 dd, 1 H, *J*(1b′,2′) = 9.3, *J*(gem) = 12.4 (Hb-1′); 4.21 m, 1 H (H-2′); 3.53 dd, 1 H, *J*(3a′,2′) = 4.5, *J*(gem) = 11.0 (Ha-3′); 3.44 dd, 1 H, *J*(3b′,2′) = 5.5, *J*(gem) = 11.0 (Hb-3′). Mass spectrum (FAB), *m*/*z* (rel.%): 211 (45) $[M + H]^+$.

Bis(2-propyl) (*S*)-9-(2-Phosphonomethoxy-3-*O*-trityloxypropyl)-2-azaadenine (*XVIa*)

A mixture of compound *XV* (2.13 g, 4.7 mmol), dimethylformamide dimethyl acetal (5 ml) and dimethylformamide (25 ml) was set aside at room temperature overnight. After evaporation and codistillation with dimethylformamide, the residue was stirred with a mixture of water (25 ml), pyridine

(25 ml) and dry ice. The solvent was evaporated, the residue codistilled with pyridine and dimethylformamide and the residue was mixed with dimethylformamide (20 ml) and bis(2-propyl) *p*-toluenesulfonyloxymethylphosphonate (*XVII*; 2.1 g, 6 mmol). The stirred mixture was cooled to −20 °C and sodium hydride (60% dispersion; 0.6 g, 15 mmol) was added. After 24 h, the mixture was neutralized with acetic acid and taken down. The residue was codistilled with toluene, dissolved in a mixture of methanol–water–ammonia (pH 10) and set aside at ambient temperature overnight. After evaporation, the residue was extracted with boiling chloroform and the product was obtained by column chromatography on silica gel (2% methanol in chloroform). Yield 1.5 g (50%) amorphous product *XVIa*. ^{1}H NMR spectrum: 8.30 s, 1 H (H-8); 7.70 s, 2 H (NH_2); 7.37 d, 6 H (arom.); 7.31 t, 6 H (arom.); 7.24 t, 3 H (arom.); 4.57 dd, 1 H, *J*(1a′,2′) = 6.8, *J*(gem) = 14.6 (Ha-1′); 4.53 dd, 1 H, *J*(1b′,2′) = 4.3, *J*(gem) = 14.6 (Hb-1′); 4.41 – 4.50 m, 2 H (POCH); 4.15 ddt, 1 H, Σ*J* = 20.1 (H-2′); 3.80 dd, 1 H, *J*(PCH) = 9.0, *J*(gem) = 13.7 (PCH_2); 3.72 dd, 1 H, *J*(PCH) = 9.3, *J*(gem) = 13.7 (PCH_2); 3.19 dd, 1 H, *J*(3a′,2′) = 4.2, *J*(gem) = 10.4 (Ha-3′); 3.00 dd, 1 H, *J*(3b′,2′) = 4.8, *J*(gem) = 10.4 (Hb-3′); 1.17 d, 3 H, *J* = 6.2 (CH_3); 1.13 d, 3 H, *J* = 6.2 (CH_3); 1.115 d, 3 H, *J* = 6.4 (CH_3); 1.06 d, 3 H, *J* = 6.0 (CH_3).

(*S*)-9-(2-Phosphonomethoxy-3-hydroxypropyl)-2-azaadenine (*XVIb*)

A mixture of compound *XVIa* (1.5 g, 2.35 mmol), acetonitrile (30 ml) and bromotrimethylsilane (3 ml) was stirred in a stoppered flask at room temperature for 24 h. The solvent was evaporated and the residue was dissolved in a mixture of 5% aqueous triethylamine (40 ml) and 80% aqueous ethanol. After evaporation, the residue was dissolved in water (30 ml) and washed three times with ether. The aqueous phase was taken down and the residue deionized on Dowex 50 X 8 (150 ml). The ammonium salt of the product was chromatographed on Dowex 1 X 2 (50 ml). After washing with water and 1 M acetic acid, the product was obtained by boiling the Dowex with 2 M acetic acid (250 ml). Evaporation, codistillation with water and crystallization from aqueous ethanol afforded 0.25 g (35%) of yellowish crystals of *XVIb*, m.p. 132 – 134 °C. For $C_8H_{13}N_6O_5P$ (304.2) calculated: 31.61% C, 4.31% H, 27.65% N, 10.19% P; found: 31.36% C, 4.27% H, 27.31% N, 10.42% P. ^{1}H NMR spectrum (D_2O): 8.63 s, 1 H (H-8); 4.66 dd, 1 H, *J*(1a′,2′) = 4.2, *J*(gem) = 14.9 (Ha-1′); 4.57 dd, 1 H, *J*(1b′,2′) = 6.3, *J*(gem) = 14.9 (Hb-1′); 3.91 m, 1 H, Σ*J* = 19.3 (H-2′); 3.81 dd, 1 H, *J*(3a′,2′) = 3.7, *J*(gem) = 12.4 (Ha-3′); 3.54 and 3.52 2 × dd, *J*(PCH) = 9.3, *J*(gem) = 12.2 (PCH_2); 3.51 dd, 1 H, *J*(3b′,2′) = 5.1, *J*(gem) = 12.4 (Hb-3′). Mass spectrum (FAB), *m*/*z* (rel.%): 305 (80) $[M + H]^+$.

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REFERENCES

1. Hockova D., Holy A., Dvorakova H.: Collect. Czech. Chem. Commun. *58* (Special Issue), 250 (1993).
2. Dvorakova H., Holy A., Votruba I., Masojidkova M.: Collect. Czech. Chem. Commun. *58*, 629 (1993).
3. Dvorakova H., Holy A., Masojidkova M., Votruba I., Balzarini J., Snoeck R., De Clercq E.: Collect. Czech. Chem. Commun. *58* (Special Issue), 253 (1993).

4. Wooley D. W., Shaw E.: J. Biol. Chem. *189*, 401 (1951).
5. Biesele J. J.: Cancer *5*, 787 (1952).
6. Shaw E., Woolley D. W.: J. Biol. Chem. *194*, 641 (1952).
7. Cappuccino J. G., George M., Merker P. C., Tarnowski G. S.: Cancer Res. *24*, 1243 (1964).
8. Montgomery J. A., Thomas H. J.: J. Med. Chem. *15*, 182 (1972).
9. Montgomery J. A., Laseter A. G., Shortnacy A. T., Clayton S. J., Thomas H. J.: J. Med. Chem. *18*, 564 (1975).
10. Holy A., Votruba I., De Clercq E.: Collect. Czech. Chem. Commun. *50*, 262 (1985).
11. Holy A., Dvorakova H., Jindrich J. in: *Antibiotics and Antiviral Compounds* (K. Krohn, H. A. Kirst and H. Maag, Eds), p. 455. VCH Weinheim, 1993.
12. Holy A. in: *Advances in Antiviral Drug Design* (E. De Clercq, Ed.) Vol. 1, p. 178. JAI Press Inc., 1993.
13. Wooley D.W., Shaw E.: J. Biol. Chem. *189*, 401 (1951).
14. Holy A.: Collect. Czech. Chem. Commun. *49*, 2148 (1984).
15. Holy A., Votruba I., De Clercq E.: Collect. Czech. Chem. Commun. *47*, 1392 (1982).
16. Holy A., Rosenberg I.: Nucleosides Nucleotides *8*, 673 (1989).
17. Budesinsky M., Masojidkova M.: Unpublished results.
18. Chenon M. T., Pugmire R. J., Grant D. M., Panzica R. P., Townsend L. B.: J. Am. Chem. Soc. *97*, 4627 (1975).
19. Kazimierczuk Z., Binding U., Seela F.: Helv. Chim. Acta *72*, 1527 (1989).
20. Kim M. J., Choi J. K.: J. Org. Chem. *57*, 1605 (1992).