SYNTHESIS AND BIOLOGICAL EFFECTS OF ACYCLIC ANALOGS OF DEAZAPURINE NUCLEOSIDES

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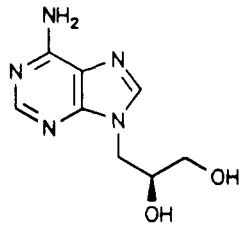
Deaza analogs of three basic types of S-adenosyl-L-homocysteine hydrolase (SAHase) inhibitors, (*S*)-DHPA (*I*), eritadenine (*II*) and AHPA (*III*), were prepared. Alkylation of 3-deazaadenine (*V*), 3-deazapurine (*VI*), 1-deazaadenine (*VII*) and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XXII*) with (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*), followed by acid hydrolysis, afforded the corresponding (*S*)-2,3-dihydroxypropyl derivatives *XVIIa* – *XIXa* and *XXV*. Reaction of *V* and *VII* with 2,3-O-cyclohexylidene-D-erythronolactone (*XXIX*) and subsequent removal of the protecting groups in an acid medium gave eritadenine analogs *XXVII* and *XXVIII*. Compounds *V* and *VII* were alkylated with bromoacetaldehyde diethyl acetal to give N-(2,2-diethoxyethyl) derivatives *XXXII* and *XXXIII* from which the substituted acetaldehyde derivatives were liberated in situ and converted into compounds *XXX* and *XXXI* by cyanohydrine reaction followed by acid hydrolysis. The alkylations were performed in dimethylformamide with sodium or cesium salts of the bases. Biological activity was observed only with 3-deazaadenine derivatives *XVIIa*, *XXVII* and *XXX*, which exhibit both enzyme-inhibitory and antiviral activities.

The so-called acyclic adenosine analogs represent large group of nucleoside analogs with interesting biological activities. These compounds differ from the natural adenine nucleosides in substitution of the sugar moiety by an aliphatic chain carrying hydroxyl or other substituents and in some cases they are very active against RNA viruses and exhibit further interesting biological effects[1].

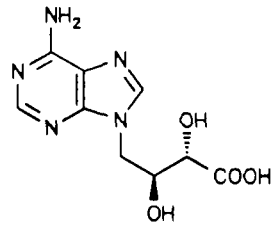
Their effects on proliferating systems[2] are explained by inhibition of the enzyme S-adenosyl-L-homocysteine hydrolase (SAHase) which catalyzes hydrolysis of S-adenosyl-L-homocysteine (SAH) to adenosine and L-homocysteine. SAH is a catabolite of S-adenosyl-L-methionine, universal donor of methyl groups in biological methylations and at the same time an inhibitor of all transmethylases[3] known so far. The inhibition of methylations of the terminal mRNA structure (so-called capping) is the probable cause of the significant antiviral effects of SAH-hydrolase inhibitors[4,5].

Systematic studies of acyclic nucleoside analogs in the adenine series have revealed three types of metabolically stable effective inhibitors of SAHase[1]: neutral compound (*S*)-DHPA (*I*)[6-8], which is a competitive inhibitor (the biological effect is specifically connected with the (*S*)-enantiomer), the natural compound D-eritadenine (*II*) and

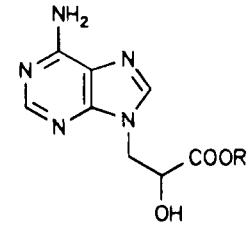
3-(adenin-9-yl)-2-hydroxypropanoic acid (AHPA (*III*)), the latter two being acidic compounds representing irreversible inhibitors (inactivators) of the enzyme[9,10].



I



II

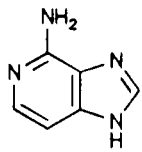


III, R = H
IV, R = alkyl

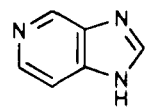
The effect of compounds containing a carboxyl group (*II* and *III*) is reduced by their polarity which limits the permeation through cell membrane. The esters of these carboxylic acids (*IV*) themselves do not inhibit SAH-hydrolase but can permeate into the cells and hydrolyze there to free carboxylic acids. Their antiviral effect is higher than that of (*S*)-DHPA (*I*) or of the parent carboxylic acids *II* and *III* (ref.[11]).

For the biological activity of the mentioned compounds an intact adenine ring is of key importance because compounds *I* – *III* act as adenosine analogs in the binding site of SAHase. It has been proved that any substitution in positions C-2, C-8 and N-6 leads to a decrease or disappearance of the biological effect[12,13].

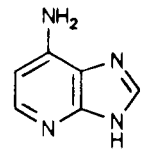
A significant modification of the purine nucleus, which practically does not alter its spatial requirements, consists in replacement of the nitrogen atom in position N-1, N-3 or N-7 by a methine grouping in the so-called deaza derivatives (e.g. 3-deazaadenine (*V*), 3-deazapurine (*VI*), 1-deazaadenine (*VII*), 7-deazaadenine (*VIIIa*)).



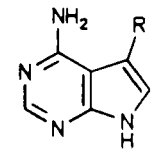
V



VI



VII



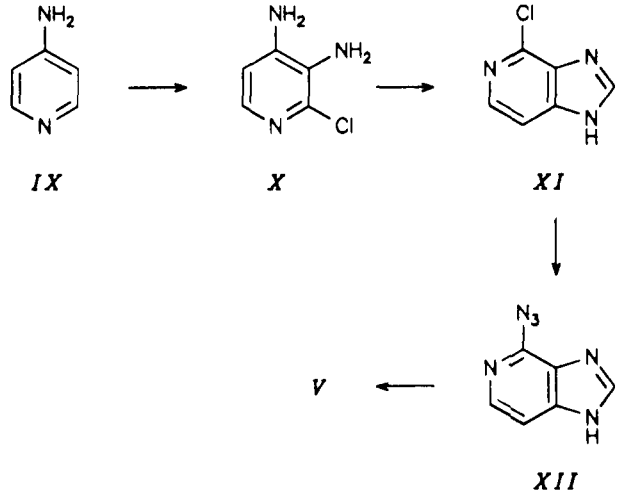
VIIIa, R = H
VIIIb, R = CN

Worth notice in the group of deaza analogs of purine nucleosides are 3-deazaadenosine[14] and 9-(β-D-arabinofuranosyl)-3-deazaadenosine[15] which are potent SAHase inhibitors (they are active against HSV-1 virus, vaccinia virus and oncogenic DNA viruses[16]); also 1-deazaadenosine[17], which inhibits blood coagulation[18], acts as adenosine aminohydrolase inhibitor[19] and exhibits also an antileukemic activity[20]. Nucleo-

sides of 7-deazaadenines (tubercidin, toyocamycin, sangivamycin) are natural antibiotics[21].

Therefore, we decided to prepare acyclic adenosine analogs of these types, containing 3-deazaadenine (*V*), 3-deazapurine (*VI*), 1-deazaadenine (*VII*) and 5-cyano-7-deazaadenine (*VIIIb*) instead of the adenine system.

3-Deazaadenine (4-amino-1*H*-imidazo[4,5-*c*]pyridine, *V*) was obtained by a modified method from 4-aminopyridine (*IX*)[16,22] via 2-chloro-3,4-diaminopyridine (*X*) which was cyclized by the action of triethyl orthoformate and the obtained 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (*XI*) was converted in the azido derivative *XII*. Hydrogenation of compound *XII* on 10% Pd/C in acetic acid afforded the desired 3-deazaadenine (*V*) (Scheme 1). This base was also prepared by an alternative five-step synthesis[22] starting from 3,4-diaminopyridine via 3-deazapurine (*VI*), its N-oxide and chloro derivative *XI*. The laboriousness and yields of both ways are similar but the first method makes use of a cheaper and more easily accessible starting compound.



SCHEME 1

Similarly to 3-deazaadenine (*V*), we also prepared 1-deazaadenine (7-amino-3*H*-imidazo[4,5-*b*]pyridine, *VII*) from 2,3-diaminopyridine via 1-deazapurine, its N-oxide and 6-nitro-1-deazapurine, making use of the published[17 - 20] methods.

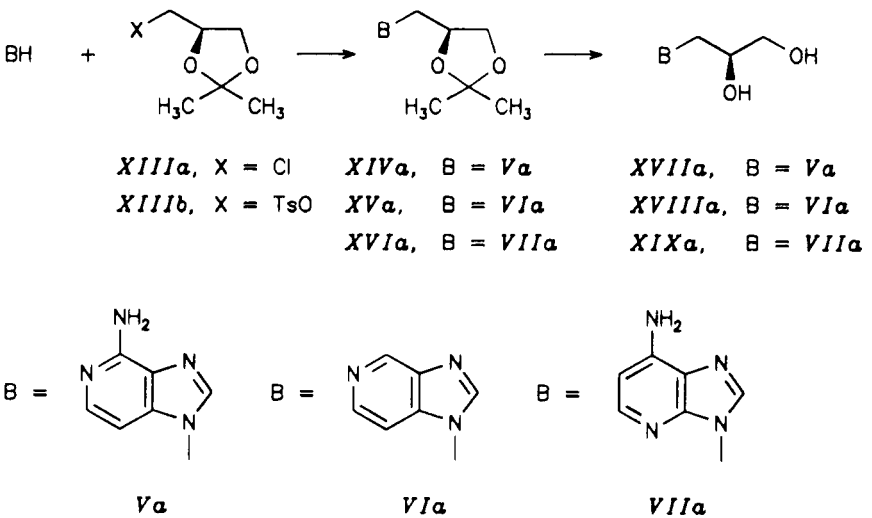
4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XXII*), the starting compound for toyocamycin analogs, was prepared according to the earlier described synthesis from tetracyanoethylene[23].

Racemic 9-(*RS*)-(2,3-dihydroxypropyl)-3-deazaadenine has already been described[24]; our interest focused on the question whether the possible inhibitory activity to SAHase is, analogously to DHPA (*I*), restricted only to the (*S*)-enantiomer. Contrary to the described procedure for the preparation of the racemic compound, based on

construction of the base from a synthon with free amino group, our approach to deazapurine derivatives of acyclic nucleosides consisted in alkylation of the base with synthons prepared earlier[25,26] for the synthesis of the corresponding adenine derivatives.

The key reaction in the preparation of the 2,3-dihydroxypropyl derivatives are N-alkylations of the corresponding heterocyclic bases with oxiranes, alkyl halides and alkyl esters of sulfonic acids (e.g. tosylates). Alkylations of adenine with alkyl halides or sulfonates are usually performed with its sodium salt obtained in situ by reaction with an equivalent amount of sodium hydride or its potassium salt formed at higher temperatures in the presence of potassium carbonate[1].

Since in some alkylations of bases with epoxides we observed a marked activating effect of cesium salts, and since a similar effect has been reported also by other authors with other bases[27], we decided to investigate in more detail the effect of the cation on the conversion and regiospecificity of alkylations of 3-deazaadenine with two types of alkylation reagents, 2,2-dimethyl-4-chloromethyl-1,3-dioxolane (*XIIIa*) and 2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*), using a series of carbonates of Na^+, K^+, Rb^+ and Cs^+ (Scheme 2).



SCHEME 2

These reactions were performed with 0.5 equivalent of M_2CO_3 (where M is the alkali metal ion) and 1.2 equivalent of the alkylation reagent relative to the base at 100 °C. All the mixtures soon became homogeneous and therefore the obtained results can be regarded as comparable with those obtained with the sodium salt, formed by preceding reaction with sodium hydride.

In both series, the reactivity decreases in the order $Cs^+ > Rb^+ > K^+ >> Na^+$. In the presence of sodium carbonate, the reaction proceeds with only very low yield. The difference between the potassium and rubidium salt consists in the equilibrium and first of all in the rate of its achievement. The most significant effect on both parameters was observed with cesium carbonate. In its presence the alkylation with both synthons proceeds with significantly higher yields than with the potassium or rubidium salt (Table I). Neither of the cations has any substantial effect on regioselectivity of the alkylation (ratio of the N^9-isomer *XIVa* to the other ones). On the basis of these observations we used in some cases cesium carbonate instead of the sodium salts.

Analogs of DHPA (*I*) were synthesized by alkylation of the corresponding base with (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*)[6]. In the case of 3-deaza-adenine (*V*), 3-deazapurine (*VI*) and 1-deazaadenine (*VII*), the alkylation was carried out with sodium salt of the base and the reaction afforded predominantly the N^9-isomers *XIVa* – *XVIa* (about 80%) which were separated from the N^7-isomers *XIVb* – *XVIb* by chromatography on silica gel. Acid hydrolysis afforded (*S*)-enantiomers *XVIIa* – *XIXa* in high yields (Scheme 2). The structural assignment to both isomers was based on comparison of their UV spectra with the literature data[16,28] and the structures were confirmed by ^{13}C NMR spectroscopy (Table II). Using the APT (attached proton test) spectra, the signals of methine and quaternary carbon atoms were distinguished and assigned on the basis of their chemical shifts. The values of chemical shifts for analogs of DHPA (*I*) observed by us are given in Table II and are in accord with the

TABLE I
The effect of cation on yield and regiospecificity of alkylation of 3-deazaadenine with two types of alkylating reagents for different reaction times

Compound	Reagent	Yield of N^9-(N^7-)isomer, %				
		2 h	4 h	8 h	14 h	20 h
Na_2CO_3	*XIIIa*	0 (0)	0 (0)	0 (0)	1 (0)	1 (0)
	XIIIb	0 (0)	0 (0)	6 (0)	13 (0)	15 (0)
K_2CO_3	*XIIIa*	9 (0)	15 (0)	27 (2)	36 (3)	46 (3)
	XIIIb	21 (3)	32 (4)	42 (5)	50 (5)	51 (5)
Rb_2CO_3	*XIIIa*	16 (0)	25 (2)	37 (3)	40 (4)	40 (4)
	XIIIb	27 (0)	39 (4)	58 (5)	62 (5)	62 (5)
Cs_2CO_3	*XIIIa*	30 (1)	41 (3)	52 (5)	57 (6)	61 (6)
	XIIIb	41 (2)	60 (3)	73 (5)	75 (5)	76 (5)
NaH	*XIIIa*	28 (1)	41 (6)	51 (8)	54 (9)	54 (9)
	XIIIb	54 (8)	54 (9)	54 (9)	55 (9)	55 (9)

published[29,30] chemical shift differences in N^9- and N^7-substituted isomers. The methine carbon atoms C-2 and C-8 in the N^9-isomers are characterized by signals in the region of δ 140. As the result of a shorter relaxation time the resonance of carbon atom C-2 exhibits higher intensity than that of carbon atom C-8. In comparison with the N^9-isomers, the spectra of N^7-isomers show a marked downfield shift of the C-3 and C-4 carbon signals and an upfield shift of the C-5, and C-6 signals whereas the resonances of C-1, C-2 and C-1′ are practically unaffected by substitution.

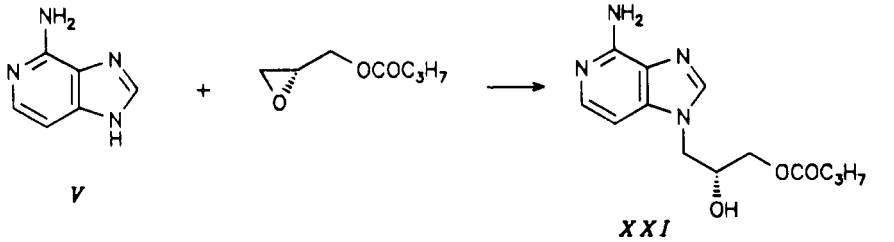
For comparison of the enzyme-inhibitory activities and synthetic utilization in the preparation of further enantiomeric derivatives[31] it was necessary to prepare also (9)-(*R*)-(2,3-dihydroxypropyl)-3-deazaadenine (*XX*). The chiral side-chain was introduced by reaction of 3-deazaadenine (*V*) with commercially accessible chiral epoxide (*R*)-glycidyl butyrate (optical purity 95%) in dimethylformamide in the presence of cesium carbonate (Scheme 3). From the reaction mixture we isolated only the N^9-isomer *XXI* which, after removal of the protecting group with sodium methoxide, was converted into the free (*R*)-DHP derivative *XX*.

In contrast to the described procedure for the preparation of the racemic 7-deaza analog of DHPA[32], which is based on utilization of a pyrrole nucleoside intermediate, our approach consists in alkylation of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XXII*) (Scheme 4). The bromo derivative was used because the substituent in position 8 prevents side-reactions and accelerates the alkylation reaction (the effect of

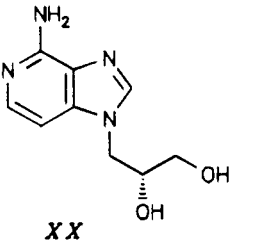
TABLE II
^{13}C NMR chemical shifts in N-substituted 3-deazaadenine, 3-deazapurine and 1-deazaadenine 2,3-dihydroxypropyl derivatives (in $(CD_3)_2SO$)[a]

Atom	*XIVa*	*XIVb*	*XVIa*	*XVIb*	*XVIIa*	*XVIIIa*	*XVIIIb*	*XIXa*
C-1	–	–	102.16	102.19	–	–	–	103.49
C-2	140.41	139.35	141.13	141.05	140.19	141.04	140.62	142.31
C-3	96.95	106.17	–	–	97.04	106.66	114.13	–
C-4	138.90	149.74	146.94	153.20	138.99	141.50	148.00	146.14
C-5	126.59	119.03	122.65	115.60	126.72	139.30	132.20	122.31
C-6	152.48	147.34	147.54	142.33	152.46	141.57	134.48	146.63
C-8	142.29	146.68	144.68	145.15	142.54	146.63	147.76	144.75
C-1′	47.02	48.50	45.28	45.72	47.85	47.73	48.06	46.19
C-2′	74.20	75.01	73.82	73.65	70.32	70.14	70.15	69.84
C-3′	66.10	65.98	66.23	66.24	63.34	63.07	63.01	63.01

[a] Chemical shifts of carbon signals of isopropylidene group in compounds *XIVa*: 109.19 26.63 25.32, *XIVb*: 109.34 26.43 25.21, *XVIa*: 108.97 26.74 25.31, *XVIb*: 109.14 26.74 25.25.

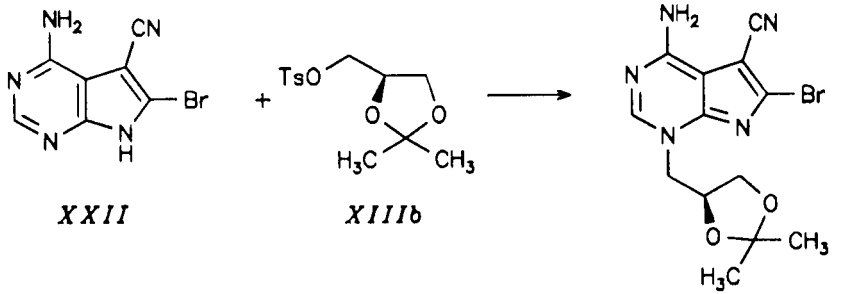


V *XXI*

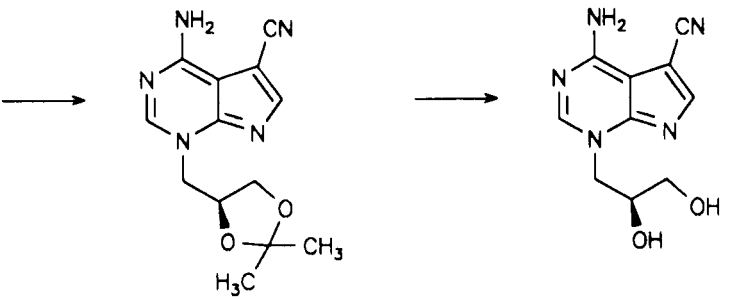


XX

SCHEME 3



XXII *XIIIb* *XXIII*

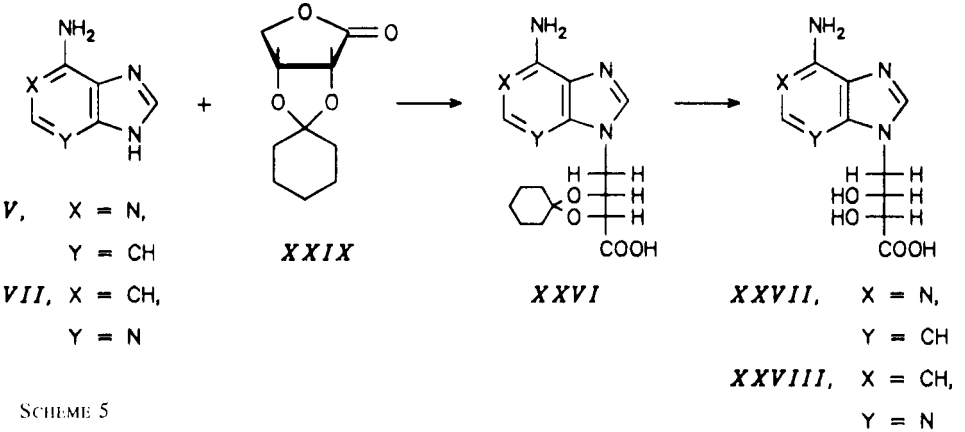


XXIV *XXV*

SCHEME 4

electron-attracting substituent)[23]. However, the alkylation of compound *XXII* with the synthon *XIIIb* in the presence of cesium carbonate, afforded predominantly the isopropylidene derivative *XXIII*, which was first hydrogenated on 10% Pd/C in the presence of magnesium oxide in methanol. The hydrogenation product *XXIV* was then converted by acid hydrolysis into free (*S*)-enantiomer *XXV*.

3-Deaza and 1-deaza analogs of eritadenine *XXVII* and *XXVIII* were prepared using an already described procedure[34,35] by reaction of 2,3-O-cyclohexylidene-D-erythronolactone (*XXIX*) with sodium (or cesium) salt of the base according to a procedure described for the synthesis of eritadenine[36]. After acid hydrolysis of the protecting groups, ion exchanger chromatography and separation on preparative HPLC, we obtained the pure N^9-isomers *XXVII* and *XXVIII* in a very poor yield (Scheme 5).



V, X = N, Y = CH
VII, X = CH, Y = N
XXIX
XXVI
XXVII, X = N, Y = CH
XXVIII, X = CH, Y = N

SCHEME 5

Analogously to the adenine derivative[9], we also prepared 3-(3-deazaadenin-9-yl)-2-hydroxypropanoic acid (*XXX*) and its 1-deaza analog *XXXI* by alkylation od deazaadenines *V* and *VII* with bromoacetaldehyde diethyl acetal. The obtained 2,2-diethoxyethyl derivatives *XXXII* and *XXXIII* were under mildly acidic conditions converted to the unstable free aldehydes from which we obtained the desired N^9-isomers *XXX* and *XXXI* by cyanohydrin reaction and subsequent acid hydrolysis (Scheme 6).

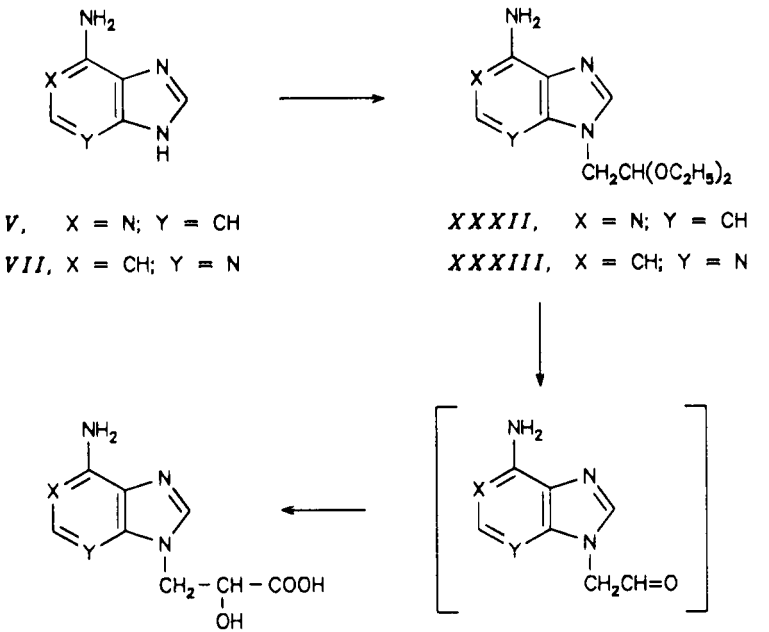
Structure of the compounds *XXVII*, *XXVIII*, *XXX* and *XXXI* were assigned on the basis of the characteristic maxima in the UV spectra which were in accord with those of the N^9-isomer of e.g. compound *XIVa* and *XVIa* (with structures determined by ^{13}C NMR).

In vitro inhibition of SAHase. For the synthesized three basic types of acyclic analogs derived from deazaadenines, i.e. analogs of 9-(*S*)-(2,3-dihydroxypropyl)adenine (*XVIIa* – *XIXa*, *XX* and *XXV*), 9-(*RS*)-(3-carboxy-2-hydroxypropyl)adenine (*XXX* and *XXXI*) and 9-(2*S*,3*S*)-(4-carboxy-2,3-dihydroxybutyl)adenine (*XXVII* and *XXVIII*), we studied

the effect on L-1210 mice leukemia cells[37] or rat liver[38] SAHase. The data given in Table III relate to enzyme catalysis of the so-called hydrolytic reaction, i.e. fission of the C–S bond in S-adenosyl-L-homocysteine under formation of adenosine and L-homocysteine. The values v_i/v_0, used for the evaluation, express the initial rates of this reaction in the presence or absence of the studied compound.

Of the 1-deazaadenine derivatives, no inhibitory activity was found for (*S*)-DHPA analog *XIXa*, D-eritadenine analog *XXVIII* and AHPA analog *XXXI*. This fact confirms that the presence of a nitrogen atom in the position 1 of the purine base is necessary for binding to the enzyme. This requirement is an absolute one, because the latter two derivatives, which in the adenine series show an extraordinarily high irreversible inactivation potency, exhibit no effect in this case.

The nitrogen atom in position 3 of the adenine ring is obviously less important for the binding to the enzyme: this follows from the fact that the 3-deazaadenine analogs of all the three mentioned types (*XVIIa*, *XXVII* and *XXX*) inhibit unequivocally SAHase and, compared with the adenine derivatives, this effect is the same or even higher. The



V, X = N; Y = CH
VII, X = CH; Y = N

XXXII, X = N; Y = CH
XXXIII, X = CH; Y = N

XXX, X = N; Y = CH
XXXI, X = CH; Y = N

SCHEME 6

literature reports an inhibitory effect of only racemic 9-(2,3-dihydroxypropyl)-3-deazaadenine[24]. Comparison of both enantiomers of this compound prepared in this study proves the enantiospecificity of interaction with the enzyme: only the (*S*)-enantiomer *XVIIa* in vitro inhibits SAHase whereas the (*R*)-enantiomer *XX* is entirely ineffective. This observation agrees with the situation in the parent adenine derivatives and proves not only that the compound is bound simultaneously to the heterocyclic base and the side-chain of the molecule but also that in the interaction with the enzyme at least three different sites in the inhibitor molecule are involved.

As expected, the inhibition effect of 3-deaza analogs of AHPA *XXX* and of D-eritadenine *XXVII* is higher than that of compound *XVIIa*. Similarly to the parent adenine derivatives, the inhibitory effect of 3-deazaDHPA (*XVIIa*) has a typical competitive character; whereas the action of 3-deazaAHPA (*XXX*) and 3-deazaeritadenine (*XXVII*) most probably consists in irreversible inactivation of SAHase.

In agreement with the expectation, also the DHP derivative of 3-deazapurine *XVIIIa* is inactive against SAHase.

The in vitro antiviral activity of the prepared deaza analogs has been in part already published by us[39]. Its determination was carried out in the Laboratory of Professor E. De Clercq, Catholic University, Leuven (Belgium). We studied systematically the effect against herpes viruses (HSV-1, HSV-2) and vaccinia virus (VV) as representatives of DNA viruses, against vesicular stomatitis virus (VSV) and in many cases also against reovirus type 1, parainfluenza virus 3, poliovirus and sindbis virus as representatives of RNA viruses, and finally against HIV and Moloney sarcoma virus (MSV) as retroviruses.

TABLE III
Inhibition of SAH-hydrolase by acyclic nucleoside analogs

Compound	Abbreviation	v_i/v_0[a]	K_i (nM)	K_i/K_m
XVIIa	3-deaza-(*S*)-DHPA	0.38	300 ± 38	0.036
XVIIIa	3-deaza-(*S*)-DHPPu	1.09	–	–
XIXa	1-deaza-(*S*)-DHPA	0.95	–	–
XX	3-deaza-(*R*)-DHPA	0.97	–	–
XXVII	3-deazaeritA	0.00	25 ± 5.0	0.003
XXVIII	1-deazaeritA	0.74	–	–
XXX	3-deazaAHPA	0.00	–	–
XXXI	1-deazaAHPA	1.18	7.6 ± 2.7	0.0009

[a] Initial rate of hydrolysis of SAH in the presence (v_i) or absence (v_0) of inhibitor; [SAH] = $4 \cdot 10^{-6}$ mol l^{-1}; [I]/[S] = 0.250.

No antiviral activity was observed with 1-deazaadenine derivatives *XIXa*, *XXVIII* and *XXXI* and toyocamycin analog *XXV*. The only heterocyclic system linked with a certain antiviral activity is again the 3-deazaadenine moiety: indications of the effect appear already with 3-deaza-(*S*)-DHPA (*XVIIa*) which is a weak SAHase inhibitor (vide supra), however, an unequivocal inhibitory activity can be observed only with 3-deaza analog of D-eritadenine (*XXVII*)[40]. As expected, this compound is active both against the VSV and DNA viruses (VV and herpes viruses, first of all VZV). Marked is the inhibition of multiplication of varicella zoster virus (VZV) and cytomegaloviruses (CMV). With VZV, no difference was observed in the sensitivity of wild strains and clinical isolates which do not induce virus-specific thymidine kinase because the probable mechanism of action, based on inhibition of methylation reactions due to interference with SAHase, does not require a preceding phosphorylation, a condition for the action of other nucleoside virostatics.

In accord with its inert behaviour to SAHase, also 9-(2,3-dihydroxypropyl)-3-deazapurine (*XVIIIa*) was inactive.

EXPERIMENTAL

Unless stated otherwise, the solvents were evaporated at 40 °C/2 kPa and the products were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected.

Thin-layer chromatography was carried out on Silufol UV 254 sheets (Kavalier, Czech Republic), preparative thin-layer chromatography on 50 × 16 × 0.3 mm layers of silica gel UV 254 (Silpearl, Votice, Czech Republic). Preparative column chromatography was performed on silica gel (Silpearl, Votice, Czech Republic). Reversed-phase chromatography was carried out on octadecyl silica gel (20 μm, Laboratorní přístroje, Praha), detection at 254 nm on a Uvicord 4701A instrument (LKB, Sweden). Preparative HPLC was performed on an Alltech 300 × 51 mm column packed with Separon SGX-RPS 10 mm; on the same type of reversed phase also analytical HPLC was carried out (column 200 × 4 mm).

Reaction mixtures were desalted on Dowex 50X8 (H^+ form): after application of the mixture, the column was first washed with water until the UV absorption of the eluate dropped to the original value and the compound was then eluted with 2.5% aqueous ammonia. Chromatography on Dowex 1X2 (acetate form) was executed in the following manner: after application, the column was washed with water until the UV absorption dropped to the original value and then the compound was eluted with a linear gradient of acetic acid or with dilute acetic acid. Concentrations of acetic acid used for the individual compounds are given in the text.

Paper electrophoreses were performed on a Whatman No. 3 MM paper at 20 V/cm (1 h) in 0.1 M triethylammonium hydrogen carbonate (TEAB). The electrophoretic mobilities given in the text (E_{Up}) are related to uridine 3'-phosphate.

UV absorption spectra were measured on a Pye–Unicam PU 8800 UV-VIS spectrophotometer (Cambridge, Great Britain) or on a Beckman DU-65 instrument.

^{1}H NMR spectra were measured on a Varian UNITY 200 spectrometer (200.01 MHz for ^{1}H) and Varian UNITY 500 (499.8 MHz for ^{1}H) in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard or in D_2O with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as internal standard. ^{13}C NMR spectra were taken on a Varian UNITY 200 instrument (50.31 MHz for ^{13}C); the spectra were referenced to the solvent signal, δ^{13}C(DMSO) = 39.7, or dioxane as an external standard, δ^{13}C(dioxane) = 66.86 for solutions in D_2O.

Optical rotations were determined on a Perkin–Elmer 141 MC A polarimeter at 20 °C.

Inhibition of SAH-hydrolase. [^{14}C]-S-Adenosyl-L-homocysteine was prepared by enzyme-catalyzed synthesis from [^{14}C]adenosine (ÚVVVR, Praha; 16 GBq mmol^{-1}) (ref.[7]). Rat liver SAH-hydrolase was purified to homogeneity according to a published procedure[38]. The basic inhibition studies (v_i/v_0) were performed in the direction of hydrolysis of SAH in the reaction mixture which contained (in total reaction volume 0.25 ml): 50 mmol l^{-1} Sörensen phosphate buffer pH 7.4, 0.1 mmol l^{-1} EDTA, 4 μmol l^{-1} [^{14}C]-SAH (1.83 MBq μmol^{-1}), 1 μmol l^{-1} compound tested, 50 μg ml^{-1} bovine serum albumine and 1.6 E.U. ml^{-1} adenosine aminohydrolase. The reaction proceeded at 37 °C for 10 min. The reaction products (SAH and inosine) were separated by paper chromatography in 2-propanol–25% ammonia–water (7 : 1 : 2) and the radioactivity in the corresponding spots was determined in a toluene scintillation cocktail on a scintillation spectrometer. The kinetic measurements (determination of K_i and K_m) were carried out according Lineweaver–Burke and Dixon and the data are mean values from three independent experiments. The constants were determined in the above-described reaction mixture with variable concentration of the substrate and the individual inhibitors.

Compounds and reagents used. 4-Aminopyridine (*IX*), 2,3-diaminopyridine, bromoacetaldehyde diethyl acetal, rubidium carbonate and cesium carbonate were Fluka products, 10% palladium on carbon was purchased from Merck, (*R*)-glycidyl butyrate from Cienzyme Fine Chemicals (England) and sodium hydride from Janssen. Dimethylformamide was dried by distillation from phosphorus pentoxide and was stored over molecular sieves.

Preparation of 3-Deazaadenine (*V*)

Fuming nitric acid (2.3 ml) was added dropwise to a vigorously stirred and cooled mixture of 4-aminopyridine (5.0 g, 55 mmol) and concentrated sulfuric acid (12.5 ml). The mixture was then stirred at room temperature for 5 h, poured on crushed ice and neutralized with aqueous ammonia. After filtration, the formed orange precipitate was crystallized from water, yield 6.0 g (79%) of 4-nitroaminopyridine, m.p. 242 – 244 °C (reported[41] m.p. 243 – 244 °C), TLC in chloroform-methanol (8 : 2) R_F 0.35.

4-Nitroaminopyridine (5.0 g) was gradually added to concentrated sulfuric acid (25 ml) with stirring and cooling. After heating to 150 °C the mixture was poured on crushed ice and made alkaline with aqueous ammonia. The separated compound was filtered and crystallized from water, yield 4.3 g (80%) of 4-amino-3-nitropyridine, m.p. 202 – 205 °C (reported[41] m.p. 200 °C), TLC in chloroform–methanol (8 : 2) R_F 0.81.

Finely ground tin(II) chloride (32 g) was gradually added to a mixture of 4-amino-3-nitropyridine (4.0 g, 28 mmol) and hot concentrated hydrochloric acid (60 ml). The mixture was heated to 90 °C for 1 h, cooled with ice and further stirred for 1 h. The white precipitate was filtered, dissolved in water and desalted on Dowex 50X8 (H^+ form, 100 ml). After elution with water the Dowex was transferred into a beaker, neutralized with 10% aqueous ammonia to pH 11, filtered and thoroughly washed with 10% aqueous ammonia. The filtrate was evaporated, the residue codistilled with ethanol (3 × 100 ml) and crystallized from ethanol, yield 2.5 g (62%) of compound *X*, m.p. 154 – 155 °C (reported[41] m.p. 162 °C), TLC in chloroform–ethanol (8 : 2) R_F 0.49.

A mixture of compound *X* (2 g, 14 mmol), triethyl orthoformate (10 ml), acetic anhydride (10 ml) and 5.7 M solution of hydrogen chloride in dimethylformamide (5 ml) was refluxed for 5 h until the starting compound disappeared (TLC in chloroform–ethanol 4 : 1, R_F 0.58). After cooling, the reaction mixture afforded the product *XI* as its crystalline hydrochloride which was deionized on Dowex 50X8 (H^+ form, 50 ml). The ammonia eluate was concentrated, the residue codistilled with ethanol (3 × 50 ml) and crystallized from ethanol, yield 1.5 g (75%) of compound *XI*, m.p. 269 – 272 °C (reported[22] m.p. 274 °C), k = 3.2 (9% acetonitrile in 0.05 M TEAB).

A stirred mixture of compound *XI* (7.0 g, 45.6 mmol), lithium azide (5.8 g, 112 mmol) and dimethylformamide (50 ml) was heated to 120 °C for 7 h to disappearance of the starting compound (TLC in chloroform–ethanol 8 : 2, R_F 0.4). After evaporation of dimethylformamide, the residue was thoroughly washed with water, filtered and washed with water, acetone and ether, yield 6.1 g (83%) of compound *XII*, m.p. > 250 °C, k = 2.3 (9% acetonitrile in 0.05 M TEAB), ^{1}H NMR and UV spectra correspond to those published in the literature[16].

Compound *XII* (5.6 g, 45 mmol) was hydrogenated in acetic acid (700 ml) on 10% Pd/C (1.4 g) at 50 °C until the starting compound disappeared (24 h, TLC in chloroform–ethanol 4 : 1, R_F 0.12). The reaction mixture was filtered while hot through a layer of Celite which was then thoroughly washed and the filtrate was concentrated. The residue (acetate) was deionized on Dowex 50X8 (H^+ form, 150 ml), the ammonia eluate was concentrated, the residue codistilled with ethanol (3 × 100 ml) and crystallized from water–ethanol (1 : 4) to give 4.5 g (75%) of compound *V*, m.p. 225 – 226 °C, k = 1.9 (9% acetonitrile in 0.05 M TEAB), ^{1}H NMR and UV spectra corresponding to the published data[16].

Alkylation of 3-Deazaadenine (*V*)

2,2-Dimethyl-4-chloromethyl-1,3-dioxolane (*XIIIa*; 0.066 g, 0.44 mmol) or 2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*; 0.12 g, 0.44 mmol) was added to a mixture of 3-deazaadenine (*V*; 50 mg, 0.37 mmol) and M_2CO_3 (M = Na, K, Rb or Cs; 0.185 mmol) or NaH (0.37 mmol) in dimethylformamide (1.5 ml). The mixture was stirred and heated under calcium chloride protecting tube. Samples were taken after 2, 4, 8, 14 and 20 h and were analyzed by HPLC in 20% acetonitrile in 0.05 M TEAB k(base) = 0.7, k(N^7-isomer) = 2.7, k(N^9-isomer) = 3.2 (Table I).

9-(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-3-deazaadenine (*XIVa*) and 7-(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-3-deazaadenine (*XIVb*)

Sodium hydride (800 mg, 20 mmol) was added to a suspension of 3-deazaadenine (*V*; 2.7 g, 20 mmol) in dimethylformamide (70 ml) and the mixture was stirred at 80 °C for 1 h under calcium chloride protecting tube. After addition of (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*; 6.3 g, 22 mmol), the mixture was heated to 100 °C (calcium chloride protection) for 20 h to disappearance of the starting compound (TLC in chloroform–ethanol 7 : 3). The solvent was evaporated, the residue codistilled with toluene (2 × 50 ml) and extracted with boiling toluene (250 ml total). The extract was concentrated in vacuo and the residue crystallized from methanol to give 1.6 g of orange crystals. Chromatography of the mother liquors on silica gel (120 g) in chloroform afforded further 0.15 g of the N^9-isomer *XIVa*; total yield 1.75 g (35%), m.p. 196 – 198 °C, R_F 0.25 (chloroform–ethanol 7 : 3). For $C_{12}H_{16}N_4O_2$ (248.2) calculated: 58.05% C, 6.49% H, 22.55% N; found: 57.70% C, 6.48% H, 22.55% N. ^{1}H NMR ($(CD_3)_2SO$): 8.02 s, 1 H (H-8 arom.); 7.68 d, 1 H (H-2 arom., J(2,3) = 5.8); 6.85 d, 1 H (H-3 arom., J(2,3) = 5.8); 6.15 brs, 2 H (NH_2); 4.37 dd, 1 H (H-1', J(1',2') = 3.5, J_g = 14.5); 4.22 dd, 1 H (H-1", J(1",2') = 7.0, J_g = 14.5); 4.37 m, 1 H (H-2', ΣJ = 22.5); 4.06 dd, 1 H (H-3', J(3',2') = 6.4, J_g = 8.8); 3.66 dd, 1 H (H-3", J(3",2') = 5.6, J_g = 8.8); 1.27 and 1.24 2 × s, 6 H (2 × CH_3). UV spectrum (pH 2): λ_{max} 261.0 nm (ε_{max} 8 800).

Precipitation from the methanolic solution with ether–light petroleum mixture afforded 0.19 g (4%) of amorphous N^7-isomer *XIVb*, R_F 0.64 (chloroform–ethanol 7 : 3). For $C_{12}H_{16}N_4O_2$ (248.2) calculated: 58.05% C, 6.49% H, 22.55% N; found: 57.87% C, 6.75% H, 22.01% N. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.11 s, 1 H (H-8 arom.); 7.68 d, 1 H (H-2 arom., J(2,3) = 5.8); 6.92 d, 1 H (H-3 arom., J(2,3) = 5.8); 5.96 brs, 2 H (NH_2); 4.68 dd, 1 H (H-1', J(1',2') = 6.0, J_g = 17.0); 4.41 dd, 1 H (H-1", J(1",2') = 7.0, J_g = 17.0); 4.41 m, 1 H (H-2', ΣJ = 24.0); 4.09 dd, 1 H (H-3', J(3',2') = 6.0, J_g = 8.8); 3.73 dd, 1 H (H-3", J(3",2') = 5.2, J_g = 8.8); 1.25 and 1.22 2 × s, 6 H (2 × CH_3). UV spectrum (pH 2): λ_{max} 290.6 nm (ε_{max} 7 600), λ_{max} 247.5 nm (ε_{max} 3 200).

9-(*S*)-(2,3-Dihydroxypropyl)-3-deazaadenine (*XVIIa*)

A solution of compound *XIVa* (1.6 g, 6.4 mmol) in 0.25 M sulfuric acid (30 ml) was allowed to stand at room temperature overnight, diluted with water (50 ml) and neutralized to pH 7.0 with saturated barium hydroxide solution. The suspension was heated to the boil, filtered through Celite which was then washed with boiling water (500 ml). The filtrate was concentrated in vacuo and the residue codistilled with ethanol (100 ml) and crystallized from 80% ethanol; yield 1.2 g of hemisulfate which was deionized on Dowex 50X8 (H^+ form). Crystallization from 80% ethanol afforded 0.62 g (47%) of compound *XVIIa*, m.p. 174 – 175 °C, R_F 0.2 (chloroform–ethanol 7 : 3), k = 1.84 (5% acetonitrile in 0.05 M TEAB), $[\alpha]_D$ −32.62°. For $C_9H_{12}N_4O_2$ (208.2) calculated: 51.92% C, 5.81% H, 26.89% N; found: 52.32% C, 5.79% H, 26.71% N. ^{1}H NMR spectrum ($(CD_3)_2SO$): 7.96 s, 1 H (H-8 arom.); 7.65 d, 1 H (H-2 arom., $J(2,3)$ = 5.8); 6.78 d, 1 H (H-3 arom., $J(3,2)$ = 5.8); 6.11 brs, 2 H (NH_2); 5.09 d, 1 H (OH-2', J = 5.4); 4.85 t, 1 H (OH-3', J = 5.0); 4.24 dd, 1 H (H-1', $J(1',2')$ = 3.5, J_g = 14.4); 4.03 dd, 1 H (H-1'', $J(1'',2')$ = 7.5, J_g = 14.4); 3.75 m, 1 H (H-2'); 3.32 m, 2 H (H-3'). UV spectrum (pH 2): λ_{max} 261.0 nm (ε_{max} 9 800); (pH 13): λ_{max} 264.0 nm (ε_{max} 10,100).

9-(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-3-deazapurine (*XVa*) and 7-(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-3-deazapurine (*XVb*)

Sodium hydride (800 mg, 20 mmol) was added to a suspension of 3-deazapurine (*VI*; 2.3 g, 20 mmol) in dimethylformamide (70 ml) and the mixture was stirred at 80 °C for 1 h (calcium chloride protecting tube). (*R*)-2,2-Dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*; 8.3 g, 29 mmol) was then added and the mixture was heated at 100 °C for total 32 h (calcium chloride protecting tube). After evaporation and codistillation with toluene (2 × 50 ml), the residue was extracted with boiling chloroform (250 ml total) and the extract was evaporated in vacuo. The residue was adsorbed onto silica gel (20 g) and chromatographed on a column of silica gel (100 g) in chloroform. The product *XVa* was eluted with chloroform–ethanol (95 : 5); yield 0.23 g (5%) of amorphous N^9-isomer *XVa*, R_F 0.43 (chloroform–methanol 7 : 3).

Further chromatography afforded 0.81 g (17%) of the N^7-isomer, also in an amorphous form, R_F 0.25 (chloroform–methanol 7 : 3). These compounds were not further characterized and were used for deblocking.

9-(*S*)-(2,3-Dihydroxypropyl)-3-deazapurine (*XVIIIa*)

A mixture of compound *XVa* (0.23 g, 1 mmol) and 0.25 M sulfuric acid (12 ml) was set aside at room temperature overnight. The reaction mixture was then applied onto Dowex 50X8 (H^+ form) and was deionized. Yield 0.13 g (47%) of oily product *XVIIIa*, k = 3.40 (2% acetonitrile in 0.05 M TEAB). For $C_9H_{11}N_3O_2$ (193.2) calculated: 55.95% C, 5.74% H, 21.74% N; found: 55.02% C, 5.38% H, 21.13% N. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.95 d, 1 H (H-6 arom., $J(6,2)$ = 1.1); 8.35 d, 1 H (H-2 arom., $J(3,2)$ = 5.7); 8.30 s, 1 H (8-H arom.); 7.65 dd, 1 H (H-3 arom., $J(2,3)$ = 5.6, $J(3,6)$ = 1.1); 5.10 br, 2 H (2 × OH); 4.43 dd, 1 H (H-1'; $J(1',2')$ = 3.5, J_g = 14.3); 4.19 dd, 1 H (H-1'', $J(1'',2')$ = 7.0, J_g = 14.3); 3.84 m, 1 H (H-2', ΣJ = 22); 3.41 dd, 1 H (H-3', $J(3',2')$ = 5.0, J_g = 11.0); 3.27 dd, 1 H (H-3'', $J(3'',2')$ = 6.5, J_g = 11.0). UV spectrum (pH 2): λ_{max} 262.4 nm (ε_{max} 4 700); λ_{max} 257.0 nm (ε_{max} 4 100); (pH 13): λ_{max} 268.0 nm (ε_{max} 2 800); λ_{max} 259.0 nm (ε_{max} 4 100).

7-(*S*)-(2,3-Dihydroxypropyl)-3-deazapurine (*XVIIIb*)

A mixture of compound *XVb* (0.81 g, 3.5 mmol) and 0.25 M sulfuric acid (40 ml) was allowed to stand at room temperature overnight. The reaction mixture was applied onto Dowex 50X8 (H^+ form) and was deionized. Yield 0.42 g (63%) of amorphous compound *XVIIIb*, k = 7.0 (2% acetonitrile in 0.05 M TEAB). For $C_9H_{11}N_3O_2$ (193.2) calculated: 55.95% C, 5.74% H, 21.74% N; found: 54.66% C, 5.62% H, 21.29% N. ^{1}H NMR spectrum ($(CD_3)_2SO$): 9.01 d, 1 H (H-6 arom., $J(6,3)$ = 1.1); 8.35 s, 1 H (8-H arom.); 8.33 d, 1 H

(H-2 arom., $J(3,2) = 5.6$); 7.66 dd, 1 H (H-3 arom., $J(2,3) = 5.6$, $J(3,6) = 1.1$); 5.14 br, 2 H (2 × OH); 4.51 dd, 1 H (H-1', $J(1',2') = 3.5$, $J_g = 14.4$); 4.26 dd, 1 H (H-1'', $J(1'',2') = 7.0$, $J_g = 14.4$); 3.87 m, 1 H (H-2', $\Sigma J = 22$); 3.42 dd, 1 H (H-3', $J(3',2') = 5.0$, $J_g = 11.0$); 3.27 dd, 1 H (H-3'', $J(3'',2') = 6.5$, $J_g = 11.0$). UV spectrum (pH 2): λ_{max} 283.5 nm (ε_{max} 7 100); λ_{max} 253.0 nm (ε_{max} 6 000); (pH 13): λ_{max} 275.2 nm (ε_{max} 6 700); λ_{max} 282.0 nm (ε_{max} 5 300).

9-(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-1-deazaadenine (*XVIa*) and 7-(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-1-deazaadenine (*XVIb*)

Sodium hydride (800 mg, 20 mmol) was added to a suspension of 1-deazaadenine (*VII*; 2.7 g, 20 mmol) in dimethylformamide (70 ml) and the mixture was stirred at 80 °C for 1 h (calcium chloride protecting tube). (*R*)-2,2-Dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*; 6.3 g, 22 mmol) was then added and the mixture was heated to 100 °C for 20 h under protection from air moisture. The hot reaction mixture was filtered through Celite, the filtrate was concentrated, the residue codistilled with toluene (3 × 50 ml), adsorbed on silica gel (20 g) and chromatographed on a column of silica gel (100 g) in chloroform. The product *XVIa* was eluted with chloroform–ethanol (98 : 2); yield 0.65 g (13%), m.p. 122 – 124 °C, R_F 0.70 (chloroform–ethanol 7 : 3), $k = 1.7$ (20% acetonitrile in 0.05 M TEAB). For $C_{12}H_{16}N_4O_2$ (248.2) calculated: 58.05% C, 6.49% H, 22.55% N; found: 57.70% C, 6.42% H, 22.17% N. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.02 s, 1 H (H-8 arom.); 7.82 d, 1 H (H-2 arom., $J(1,2) = 5.4$); 6.36 d, 1 H (H-1 arom., $J(1,2) = 5.4$); 4.25 m, 2 H (H-1'); 4.46 brq, 1 H (H-2', $\Sigma J = 17.5$); 4.00 dd, 1 H (H-3', $J(3',2') = 6.5$, $J_g = 9.0$); 3.72 dd, 1 H (H-3'', $J(3'',2') = 5.5$, $J_g = 9.0$); 1.29 and 1.23 s, 6 H (2 × CH_3). UV spectrum (pH 2): λ_{max} 281.0 nm (ε_{max} 14,300), (pH 2): λ_{max} 262.0 nm (ε_{max} 11,500).

Further elution gave the N^7-isomer *XVIb* (0.35 g, 7%), R_F 0.82 (chloroform–ethanol 7 : 3) as an oil which could not be purified to analytical purity. Its structure was proved by ^{13}C NMR spectrum (Table II). UV spectrum (pH 2): λ_{max} 276.5 nm, (pH 13): λ_{max} 278.4 nm.

9-(*S*)-(2,3-Dihydroxypropyl)-1-deazaadenine (*XIXa*)

A mixture of compound *XVIa* (0.6 g, 2.4 mmol) and 0.25 M sulfuric acid (30 ml) was set aside at room temperature overnight. The reaction mixture was applied on Dowex 50X8 (H^+ form) and deionized in the usual manner (vide supra). After evaporation of the ammonia eluate in vacuo, the residue was codistilled with ethanol (100 ml) and crystallized from ethanol, affording 0.46 g of compound *XIXa*, m.p. 101 – 102 °C; $k = 3.70$ (6% acetonitrile in 0.05 M TEAB), $[\alpha]_D$ −26.50°. For $C_9H_{12}N_4O_2 \cdot H_2O$ (226.2) calculated: 47.79% C, 6.24% H, 27.46% N; found: 48.53% C, 5.84% H, 25.04% N. ^{1}H NMR spectrum (D_2O): 8.00 s, 1 H (H-8 arom.); 7.84 d, 1 H (H-2 arom., $J(1,2) = 5.4$); 6.50 d, 1 H (H-1 arom., $J(1,2) = 5.4$); 4.16 m, 3 H (H-1' and H-2'); 3.65 m, 2 H (H-3'). UV spectrum (pH 2): λ_{max} 281.0 nm (ε_{max} 17,500), λ_{max} 260.0 nm (ε_{max} 13,800); (pH 13): λ_{max} 275.0 nm (ε_{max} 12,000), λ_{max} 262.0 nm (ε_{max} 14,500).

9-(*R*)-(2,3-Dihydroxypropyl)-3-deazaadenine (*XX*)

A mixture of 3-deazaadenine (*V*; 1.34 g, 10 mmol), cesium carbonate (100 mg), dimethylformamide (25 ml) and (*R*)-glycidyl butyrate (1.73 g, 12 mmol) was heated to 100 °C for 5 h (calcium chloride protecting tube). After evaporation of the solvent and codistillation of the residue with toluene (3 × 50 ml), the formed 3'-butyrate *XXI* was directly deblocked by treatment with 0.1 M sodium methoxide (50 ml) at room temperature for 24 h. The reaction mixture was then neutralized with Dowex 50X8 (H^+ form) and adjusted to pH 12 with triethylamine. After removal of the Dowex by filtration, washing with methanol and evaporation in vacuo, the residue was deionized on Dowex 50X8 (H^+ form) and crystallized from 80% ethanol; yield 0.87 g (42%), m.p. 250 °C; $k = 3.3$ (4% acetonitrile in 0.05 M TEAB), $[\alpha]_D$ +30.66°. For $C_9H_{12}N_4O_2$ (208.2) calculated: 51.92% C, 5.81% H, 26.89% N; found: 51.34% C, 5.60% H, 26.35% N. ^{1}H NMR

spectrum ($(CD_3)_2SO$): 7.97 s, 1 H (H-8 arom.); 7.66 d, 1 H (H-2 arom., $J(2,3)$ = 5.8); 6.80 d, 1 H (H-3 arom., $J(3,2)$ = 5.8); 6.09 brs, 2 H (NH_2); 4.80 br, 2 H (2 × OH); 4.27 dd, 1 H (H-1', $J(1',2')$ = 3.7, J_g = 14.0); 4.03 dd, 1 H (H-1", $J(1",2')$ = 7.0, J_g = 14.0); 3.76 m, 1 H (H-2', ΣJ = 22.0); 3.42 dd, 1 H (H-3", $J(3',2')$ = 4.8, J_g = 11.0); 3.26 dd, 1 H (H-3", $J(3",2')$ = 6.5, J_g = 11.0). ^{13}C NMR spectrum ($(CD_3)_2SO$): 152.46 (C-6), 142.54 (C-8), 140.19 (C-2), 138.99 (C-4), 126.72 (C-5), 97.04 (C-3), 70.32 (C-2'), 63.34 (C-3'), 47.85 (C-1'). UV spectrum (pH 2): λ_{max} 263.0 nm (ε_{max} 9 300); (pH 13): λ_{max} 266.5 nm (ε_{max} 9 500).

4-Amino-6-bromo-5-cyano-1-[((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl]pyrrolo-[2,3-*d*]pyrimidine (*XXIII*)

A mixture of compound *XXII* (1.2 g, 5 mmol), cesium carbonate (0.82 g, 2.5 mmol), (*R*)-2,2-dimethyl-4-tosyloxymethyl-1,3-dioxolane (*XIIIb*; 6.9 g, 5.5 mmol) and dimethylformamide (30 ml) was heated to 100 °C (calcium chloride protecting tube) to disappearance of the starting compound (48 h; TLC in chloroform–ethanol 9 : 1). After evaporation and codistillation with toluene (2 × 50 ml), the residue was extracted with boiling chloroform, the extract was evaporated and the residue chromatographed on a column of silica gel (120 g) in chloroform. The product was eluted with chloroform–ethanol (99 : 1) and crystallized from methanol to give 0.67 g (41%) of compound *XXIII*, R_F 0.53 (chloroform–ethanol 9 : 1). For $C_{13}H_{14}N_5O_2Br$ (352.1) calculated: 44.34% C, 4.01% H, 19.88% N, 22.69% Br; found: 44.43% C, 3.82% H, 19.71% N, 22.45% Br. ^1H NMR spectrum ($(CD_3)_2SO$): 8.22 s, 1 H (H-2 arom.); 6.98 brs, 2 H (NH_2); 4.38 dd, 1 H (H-1', $J(1',2')$ = 4.6, J_g = 14.2); 4.28 dd, 1 H (H-1", $J(1",2')$ = 8.3, J_g = 14.2); 4.53 m, 1 H (H-2'); 4.04 dd, 1 H (H-3', $J(3',2')$ = 6.35, J_g = 8.8); 3.82 dd, 1 H (H-3", $J(3",2')$ = 4.6, J_g = 8.8); 1.32 and 1.20 s, 2 × 3 H (2 × CH_3). UV spectrum (methanol): λ_{max} 286.5 nm (ε_{max} 15,000), λ_{max} 262.5 nm (ε_{max} 9 800).

4-Amino-1-[(*S*)-2,3-dihydroxypropyl]-5-cyanopyrrolo[2,3-*d*]pyrimidine (*XXV*)

A mixture of compound *XXIII* (0.6 g, 1.9 mmol), 10% Pd/C (1.0 g), magnesium oxide (1.0 g) and methanol (for UV spectroscopy; 50 ml) was stirred in a hydrogen atmosphere until the starting compound disappeared (6 h, TLC in chloroform–ethanol 9 : 1). The reaction mixture was filtered through a layer of Celite which was then thoroughly washed with hot methanol, and the filtrate was concentrated. The amorphous product *XXIV* (R_F 0.45) was dissolved without further purification in 0.25 M sulfuric acid (30 ml) and allowed to stand at room temperature overnight. The reaction mixture was then neutralized with saturated barium hydroxide solution to pH 7. The suspension was taken to the boil, filtered through Celite which was then washed with boiling water (250 ml). The filtrate was concentrated and the residue codistilled with ethanol (50 ml) and crystallized from methanol. Yield 0.17 g (35%) of compound *XXV*, m.p. 208 – 210 °C; k = 2.80 (8% acetonitrile in 0.05 M TEAB). For $C_{10}H_{11}N_5O_2 . H_2O$ (251.2) calculated: 47.80% C, 5.21% H, 22.29% N; found: 47.27% C, 5.37% H, 22.08% N. ^1H NMR spectrum ($(CD_3)_2SO$): 8.28 and 7.83 2 × s, 2 H (H-2 and H-8 arom.); 7.50 br, 2 H (NH_2); 5.24 d, 1 H (OH-2', J = 5.4); 4.98 t, 1 H (OH-3', J = 5.9); 4.60 dd, 1 H (H-1', $J(1',2')$ = 3.2, J_g = 13.2); 4.09 dd, 1 H (H-1", $J(1",2')$ = 8.5, J_g = 13.2); 3.93 m, 1 H (H-2', ΣJ = 28.0); 3.30 2 × dt, 2 H (H-3', overlapped with water); after exchange with CH_3COOD: 3.91 m, 1 H (H-2', ΣJ = 22.7); 3.42 dd, 1 H (H-3', $J(3',2')$ = 4.9, J_g = 11.2); 3.29 dd, 1 H (H-3", $J(3",2')$ = 6.1, J_g = 11.2). UV spectrum (pH 2): λ_{max} 280.5 nm (ε_{max} 8 200); (pH 7): λ_{max} 275.5 nm (ε_{max} 8 150), λ_{max} [illegible]5 nm (ε_{max} 8 400); (pH 12): λ_{max} 277.0 nm (ε_{max} 8 100), λ_{max} 258.0 nm (ε_{max} 7 700).

(2*S*,3*S*)-4-(3-Deazaadenin-9-yl)-2,3-dihydroxybutanoic Acid (3-Deazaaristeromycin, *XXVII*)

A mixture of 3-deazaadenine (*V*; 1.0 g, 7.5 mmol), dimethylformamide (30 ml) and 60% sodium hydride dispersion (0.3 g, 7.5 mmol) was stirred at 80 °C for 1 h (calcium chloride protecting tube). 2,3-O-Cyclohexylidene-D-erythronolactone (*XXIX*; 3.7 g, 19 mmol) was added and the mixture was heated at 125 °C until the starting compound disappeared (monitoring by electrophoresis, 30 h). After evaporation in vacuo,

the residue was codistilled with toluene and refluxed with 85% formic acid (50 ml) for 3 h. The formic acid was evaporated *in vacuo*, the crude mixture was deionized on Dowex 50X8 (H^+ form) and chromatographed on a column of Sephadex A-25 (200 ml). Elution with 0.02 M TEAB removed the unreacted base and gradient elution (0.02 to 0.2 M of the same buffer; à 1 l) afforded the desired product which was converted to the free acid on Dowex 1X2 (acetate). Codistillation with water and ethanol and crystallization from 80% ethanol afforded 0.15 g (7%) of the N^9-isomer *XXVII*, m.p. > 250 °C, k = 0.71 (8% acetonitrile in 0.05 M TEAB), $[\alpha]_D$ +8.1°, E_{Up} = 0.4. For $C_{10}H_{12}N_4O_4 \cdot 2\,H_2O$ (288.2) calculated: 41.67% C, 5.60% H, 19.43% N; found: 42.25% C, 4.54% H, 22.04% N. ^{1}H NMR spectrum (D_2O + NaOD): 8.06 s, 1 H (H-8 arom.); 7.73 d, 1 H (H-2 arom., J(2,3) = 6.0); 6.96 d, 1 H (H-3 arom., J(2,3) = 6.0); 4.20 m, 4 H (N–CH_2 and 2 × O–CH); ^{13}C NMR spectrum (D_2O): 178.97 (C-4'), 151.72 (C-6), 144.21 (C-8), 139.75 (C-2), 139.58 (C-4), 134.55 (C-5), 101.36 (C-3), 75.51 (C-3'), 71.81 (C-2'), 47.82 (C-1'). UV spectrum (pH 2): λ_{max} 261.2 nm (ε_{max} 10,400); (pH 13): λ_{max} 264.0 nm (ε_{max} 10,600).

(2*S*,3*S*)-4-(1-Deazaadenin-9-yl)-2,3-dihydroxybutanoic Acid (1-Deazaeritadenine, *XXVIII*)

A stirred mixture of 1-deazaadenine (*VII*; 0.5 g, 0.37 mmol), dimethylformamide (20 ml), cesium carbonate (0.6 g, 1.86 mmol) and 2,3-O-cyclohexylidene-D-erythronolactone (*XXIX*; 2.2 g, 11.2 mmol) was heated at 125 °C (calcium chloride protecting tube) until the starting compound disappeared (14 h, monitored by electrophoresis). After evaporation of the solvent, the residue was codistilled with toluene and then refluxed with 85% formic acid (25 ml) for 6 h. The formic acid was evaporated in vacuo, the crude mixture was deionized on Dowex 50X8 (H^+ form) and chromatographed on a column of Dowex 1X2 (acetate form, 80 ml). Elution with water removed the unreacted base and gradient elution with 0 to 0.2 M acetic acid (à 1 l) afforded an 8 : 2 mixture of the N^9- and N^7-isomers (HPLC, 6% acetonitrile in 0.05 M TEAB) which were separated by preparative HPLC in water. Crystallization from 80% ethanol (with addition of ether to turbidity) afforded 0.10 g (11%) of N^9-isomer *XXVIII*, m.p. 250 °C, k = 0.8 (6% acetonitrile in 0.05 M TEAB), E_{Up} = 0.57. For $C_{10}H_{12}N_4O_4 \cdot 2\,H_2O$ (288.2) calculated: 41.67% C, 5.60% H, 19.43% N; found: 42.25% C, 5.54% H, 19.04% N. ^{1}H NMR spectrum (D_2O + NaOD): 8.10 s, 1 H (H-8 arom.); 7.96 d, 1 H (H-2 arom., J(2,1 = 5.6); 6.62 d, 1 H (H-1 arom., J(2,1) = 5.6); 4.30 – 4.36 m, 3 H and 4.16 d, 1 H (N–CH_2 and 2 × O–CH, undistinguished). UV spectrum (pH 2): λ_{max} 283.0 nm (ε_{max} 14,700), λ_{max} 264.0 nm (ε_{max} 11,300); (pH 13): λ_{max} 277.0 nm (ε_{max} 9 700), λ_{max} 263.0 nm (ε_{max} 11,900). Mass spectrum: 253 (M + H).

9-(2,2-Diethoxyethyl)-3-deazaadenine (*XXXII*)

A mixture of 3-deazaadenine (*V*; 1.0 g, 7.5 mmol), dimethylformamide (30 ml) and 60% sodium hydride dispersion (0.3 g, 7.5 mmol) was stirred at 80 °C for 1 h (calcium chloride protecting tube). Bromoacetaldehyde diethyl acetal (1.7 ml, 11 mmol) was then added and the mixture was heated at 80 °C until the starting compound disappeared (26 h). After evaporation, the residue was codistilled with toluene and taken up in boiling chloroform. The chloroform was evaporated and the residue was column chromatographed on silica gel (50 g) to give 0.4 g (21%) of compound *XXXII*, m.p. 154 – 155 °C, R_F 0.74 (methanol–chloroform 3 : 7), k = 1.2 (50% acetonitrile in 0.05 M TEAB). For $C_{12}H_{18}N_4O_2$ (250.3) calculated: 57.59% C, 7.25% H, 22.38% N; found: 57.85% C, 7.22% H, 21.82% N. ^{1}H NMR spectrum ($(CD_3)_2SO$): 8.00 s, 1 H (H-8 arom.); 7.06 d, 1 H (H-2 arom., J(2,3) = 5.9); 6.84 d, 1 H (H-3 arom., J(3,2) ≡ 5.9); 6.16 brs, 2 H (NH_2); 4.24 d, 2 H (H-1', J(1',2') = 5.0); 4.74 d, 1 H (H-2', J(2',1') = 5.0); 3.62 dq, 2 H (O–CH_2, J(CH_2,CH_3) = 7.0, J_g = 9.6); 3.40 dq, 2 H (O–CH_2, J(CH_2,CH_3) = 7.0, J_g = 9.6); 1.02 t, 6 H (2 × CH_3). UV spectrum (pH 2): λ_{max} 261.4 nm (ε_{max} 10,100); (pH 13): λ_{max} 264.7 nm (ε_{max} 10,200).

(*RS*)-3-(3-Deazaadenin-9-yl)-2-hydroxypropanoic Acid (*XXX*)

A mixture of compound *XXXII* (0.35 g, 1.4 mmol) and 0.25 M sulfuric acid (15 ml) was heated at 70 °C for 9 h. After cooling to −5 °C and neutralization with 2 M sodium hydroxide, sodium cyanide (0.5 g, 10 mmol) was added with stirring and cooling. The mixture was then rapidly neutralized with acetic acid to pH 6 – 6.5, stirred at 0 °C for 2 h and at room temperature overnight. Concentrated hydrochloric acid (4 ml) was added and the mixture was refluxed for 3 h. After evaporation and codistillation with water, the residue was deionized on Dowex 50X8 (H^+ form, 100 ml) and the crude product was purified on Dowex 1X2 (acetate form, 100 ml). The column was washed with water and the product was eluted with a linear gradient of acetic acid (0 – 0.5 mol l^{-1}, à 1 l). The product-containing fractions were evaporated, the residue codistilled with water and converted to the sodium salt on Dowex 50X8 (Na^+ form); yield 0.14 g (38%) of compound *XXX*, k = 1.6 (4% acetonitrile in 0.05 M TEAB), E_{Up} = 0.46. For $C_9H_9N_3O_3Na$. H_2O (262.2) calculated: 41.22% C, 4.23% H, 21.53% N; found: 41.41% C, 4.13% H, 20.90% N. ^1H NMR spectrum ($(CD_3)_2SO$): 7.92 s, 1 H (H-8); 6.00 brs, 2 H (NH_2); 7.62 d, 1 H (H-2, J(2,3) = 5.8); 6.79 d, 1 H (H-3, J(3,2) = 5.8); 4.53 br, 1 H (OH); 4.34 dd, 1 H (H-1', J(1',2') = 3.2, J_g = 14.2); 4.13 dd, 1 H (H-1", J(1",2') = 6.3, J_g = 14.2); 3.82 dd, 1 H (H-2', ΣJ = 9.5). UV spectrum (pH 2): λ_{max} 261.4 nm (ε_{max} 7 200); (pH 13): λ_{max} 265.7 nm (ε_{max} 7 100).

(*RS*)-3-(1-Deazaadenin-9-yl)-2-hydroxypropanoic Acid (*XXXI*)

A stirred mixture of 1-deazaadenine (*VII*; 0.3 g, 2.2 mmol), dimethylformamide (11 ml), cesium carbonate (0.36 g, 1.1 mmol) and bromoacetaldehyde diethyl acetal (0.6 ml, 0.79 g, 4.4 mmol) was heated at 135 °C (calcium chloride protecting tube) until the starting compound disappeared (6 h). After evaporation, the residue was codistilled with toluene and taken up in boiling chloroform. After evaporation, the residue was chromatographed on a column of silica gel (60 g) in chloroform. Chloroform eluted the N^7-isomer (R_F 0.71; 20 mg) whereas elution with chloroform–ethanol (99 : 1) gave the N^9-isomer *XXXIII* (0.24 g, 44%), R_F 0.55 (chloroform–ethanol 9 : 1). The structure of the isomers was confirmed by their UV spectra (vide infra). The assumed N^9-substituted derivative was used directly in the next reaction step.

A mixture of compound *XXXIII* (0.24 g, 1.0 mmol), water (5 ml) and concentrated hydrochloric acid (0.15 ml) was heated at 80 °C for 5 h and at 100 °C for 1 h to about 90% conversion (TLC in chloroform–ethanol 85:15, R_F of the product 0.46). The mixture was cooled to −5 °C and sodium cyanide (0.27 g, 5.6 mmol) was added with stirring and cooling, followed by acetic acid (about 0.2 ml) to neutral reaction. The mixture was then stirred at 0 °C for 2 h and at room temperature overnight. Concentrated hydrochloric acid (4 ml) was added and the mixture was refluxed for 3 h. After evaporation and codistillation with water, the residue was deionized on Dowex 50X8 (H^+ form, 50 ml) and the crude product was chromatographed on Dowex 1X2 (acetate form, 50 ml). The column was washed with water and then the product was eluted with a linear gradient of acetic acid (0 – 0.5 mol l^{-1}, à 1 l). The product-containing fractions were evaporated and the residue was codistilled with water and crystallized from 80% ethanol to give 0.14 g (27%) of compound *XXXI*, m.p. 248 – 249 °C, k = 2.1 (2% acetonitrile in 0.05 M TEAB), E_{Up} = 0.61. For $C_9H_{10}N_4O_3$. H_2O (240.2) calculated: 45.00% C, 5.03% H, 23.31% N; found: 45.55% C, 4.73% H, 24.03% N. ^1H NMR spectrum (D_2O + NaOD): 8.04 s, 1 H (H-8, arom.); 7.91 d, 1 H (H-2, J(2,1) = 5.6); 6.55 d, 1 H (H-1, J(1,2) = 5.6); 4.55 dd, 1 H (H-1', J(1',2') = 3.4, J_g = 14.4); 4.32 dd, 1 H (H-1", J(1",2') = 7.8, J_g = 14.4); 4.41 dd, 1 H (H-2', J(2',1') = 3.4, J(2',1") = 7.8). UV spectrum (pH 2): λ_{max} 283.0 nm (ε_{max} 15,500), λ_{max} 264.0 nm (ε_{max} 11,500); (pH 13): λ_{max} 277.0 nm (ε_{max} 9 800), λ_{max} 264.0 nm (ε_{max} 11,900).

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